BULLETIN No. 175 MAY, 1917

MASSACHUSETTS AGRICULTURAL EXPERIMENT STATION

Mosaic Disease of Tobacco

By G. H. CHAPMAN

This bulletin deals with the mosaic disease of tobacco, including a brief review of the work of previous investigators. It also gives results obtained at this station relating to the cause, reaction and control of the disease. It is shown that more than 80 per cent. of field infection may be traced originally to the seed bed. Specific methods for control are recommended.

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PUBLICATION OF THIS DOCUMENT
APPROVED BY THE
SUPERVISOR OF ADMINISTRATION.

BULLETIN No. 175.

DEPARTMENT OF BOTANY.

MOSAIC DISEASE OF TOBACCO.[1]

BY G. H. CHAPMAN.

INTRODUCTION.

The observations and conclusions reported in the following pages are the results of several years of more or less continuous investigation on the part of the writer, and deal with the probable causes, occurrence, appearance and methods of control of this well-known disease of tobacco and related plants. Enough has been accomplished so that it is believed wise to add still another paper to the already long list of literature which has been published on this disease. During the time in which these experiments have been in progress much new literature has appeared dealing with this subject, some of which has helped the writer by verifying his results and by bringing out new facts concerning the disease; but, on the other hand, some of the work appears to have been done in a hasty manner, and possibly erroneous conclusions drawn in some cases, thus adding to the large amount of confusing subject-matter which has to do with this disease. The experiments carried on by the writer were begun in a general way in 1907, and have been repeated several times during the years subsequent to that date, new lines of investigation both in the field and laboratory having been added as occasion demanded. Some considerable time has been spent in verifying the results obtained by other recent investigators, and an attempt has been made to gather together in a broad, general way, as well as in detail, all the reliable information possible about this interesting disease, as well as to bring out new facts in regard to it. More attention has been given to the biochemical aspects of the problem than has heretofore been done by investigators.

[1] Also presented in part to the faculty of the graduate school of the Massachusetts Agricultural College, June, 1916, as a major thesis in partial fulfillment of the requirements for the degree of doctor of philosophy.

Historical Summary.

In the following paragraphs is given a brief résumé of the more important work done on the mosaic disease of tobacco up to the present time, and as an excellent critical review of the literature, etc., up to 1902 is given by A. F. Woods[1] in his work on the subject, the same is quoted in full below. He states: —

Adolph Mayer[2] was the first to make a careful study of the trouble. He demonstrated that it could not be caused by an insufficient supply of mineral nutrients. He found as much nitrogen, potassium salts, phosphates, calcium and magnesium present in the soils and plants where the disease occurred as in the soils where the disease did not occur. He also found that the trouble was apparently distributed over the field without regard to the soil conditions.

Since tobacco requires much lime, liming the soil was tried, but the disease was not prevented thereby. Mayer further kept hotbeds in some cases rather moist, in others dry, and then again, richly or poorly manured with nitrogen; but in no case could he determine that the conditions in question caused the disease. He also found that variations in the temperature of the hotbeds apparently had no effect; neither did crowding, which produced partial etiolation, appear to have any effect on the disease. Seeds from flowers in which self-fertilization was prevented he found to be just as susceptible to the disease as seeds produced without such precautions, but on the soil on which the disease had once appeared it was again produced. According to his observation, also, the trouble was not often found on soil used for the first time for tobacco. He further proved that the juice of the diseased leaves injected with the juice of healthy plants did not develop the disease. He was not able to produce it by injecting diseased juice into other solanaceous plants. Where the diseased juice was injected into tobacco the same trouble developed in from ten to eleven days. Heating to 60° C. did not destroy the infectious substance; at 65° to 75° it was attenuated, and at 80° it was killed.

After Mayer had shown the absence of animal and fungous parasites he supposed bacteria to be the cause of the disease, but all his efforts with bacteria cultivated from the surface of diseased leaves, and also with different mixtures of bacteria, failed to produce it. Nevertheless, he thought that there must be certain pathogenic bacteria present in those soils in which the disease appeared, and therefore proposed to change the soil in the hotbeds and to devote the fields where tobacco had been cultivated to other crops. He also recommended the use of mineral rather than organic manures.

These general results were confirmed by several subsequent investigators. Not, however, till Beijerinck[3] took hold of the question was much of importance added to our knowledge of the malady. He proved the absence of bacteria in the development of the disease. He showed that the juice of the plant filtered through Chamberland filters, while remaining perfectly clear and free from bacteria, still retained the power of infection. A small drop of it injected hypodermically into the growing bud was sufficient to give the plant the disease. He found that only dividing (meristematic) cells can become diseased. Diseased tissue kept its infectious qualities even after drying, and retained its injurious properties in the

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[1] Woods, A. F.: Observations on the Mosaic Disease of Tobacco. U. S. D. A., Bur. Plant Ind., Bul. No. 18 (1902).

[2] Mayer, Adolph: Über die Mosaikkrankheit des Tabaks. Landw. Versuchsstation, 32: 451–467 (1886). Review of the same article in Journ. of Mycology, 7: 382–385 (1894).

[3] Beijerinck, M. W.: Verhandelingen der Koninklijke Akademie van Wetenschappen te Amsterdam. Deel 6: No. 5. See also Centb. f. Bakt., Par., etc., II: 5: 27–33 (1899).

soil during the winter. Weak solutions of formalin did not kill the virus, but heating to boiling point did. Fresh, unfiltered juice was more effective than an equal amount of filtered juice. He found that soil around diseased plants may infect the roots of healthy plants, but he did not determine whether direct transference is possible through healthy root surfaces, or whether insects, by injuring the roots, favored infection. He defines the milder form of the disease as a suffering of the chlorophyll bodies. Later a general disease of the plasmatic contents of the cells sets in.

In field conditions as a final stage the swollen green areas become marked with small dead spots, but these did not appear on plants grown under glass. Under certain conditions he observed that plants apparently recover from the disease; *i.e.*, the new growth appeared to recover. He found that the infective material, whatever it might be, could be transported through considerable distances in the plant, but could cause the disease only in the dividing cells. He assumed the virus to be a non-corpuscular, fluid-like material, which had the power of growth when in contact, in a sort of symbiotic way, with the growing cells, — "a living fluid contagium."

Shortly after Beijerinck's paper, Sturgis [1] published a critical review of the work done on the disease up to that time, with numerous valuable results and observations made in Connecticut, where the trouble is known as "calico" or "mottled top."

The results obtained by Sturgis and observations made by him on tobacco in Connecticut bore out the statements of other careful and critical workers, and greatly cleared up the field for further investigation. He came to the conclusion that on close, clayey soils the disease may be more abundant than on an open, porous soil. The disease is not contagious, but he could not state definitely as to its infectiousness; it is not caused by fungi, nematodes or parasitic insects, and the facts observed by him were not favorable to the theory of bacterial origin. He also came to the conclusion that the disease is not inherent in the seed, and looked upon it as a purely physiological trouble brought about by sudden interruptions of the normal plant metabolism. Koning, [2] in his work, verified much of the work of Beijerinck and Mayer, and Woods [3] later verified the work of these investigators and pointed out that in the diseased leaves there was an excess or excessive activity on the part of an enzyme belonging to the oxidases, and that the power of oxidation in the cells was inversely proportional to the amount of chlorophyll present, using the color as a basis of comparison. He also pointed out that there was a marked structural difference between the cells of the dark green and light green areas, and proved to his own satisfaction that the light green areas are the truly diseased portions, a fact that will be referred to later in this paper. In a later careful investigation of the disease Woods [4] arrived at the following conclusions, which were a great stride forward in our understanding of some phases of this baffling disease. He states: —

[1] Sturgis, W. A.: Mosaic Disease of Tobacco. Conn. Agr. Exp. Sta. Rept., 250–254 (1898).

[2] Koning, C. J.: Die Flecken oder Mosaikkrankheit des hollandischen Tabaks. Zeitschrift für Pflanzenkr., 9: 65–80.

[3] Woods, A. F.: Inhibiting Action of Oxidase on Diastase. Science, n. s., No. 262, 17–19.

[4] Woods, A. F., *loc. cit.*

The disease is not due to parasites of any kind, but is the result of defective nutrition of the young dividing and rapidly growing cells, due to a lack of elaborated nitrogenous reserve food accompanied by an abnormal increase in the activity of oxidizing enzymes in the diseased cells. The unusual activity of the enzyme prevents the proper elaboration of the reserve food, so that a plant once diseased seldom recovers. On the decay of the roots, leaves and stems of both healthy and diseased plants, the enzyme in question is liberated and remains active in the soil. The enzyme is very soluble in water and appears to pass readily through plant membranes. If the young plants take it up in sufficient quantity to reach the terminal bud, they become diseased in the characteristic way. Under field conditions there is little danger from infection in this manner, but in the seed bed the danger is much greater on account of the greater susceptibility of the young plants to the disease, and the greater amount of free oxidizing enzymes likely to be in the soil due to the decay of the roots and plants. New or steam sterilized soil should therefore be used for the seed bed.

I have shown that transplanting, especially when the roots are injured, may produce the disease. Great care must, therefore, be taken not to injure the roots in this process or in the subsequent cultivation, or to check the growth of the plants.

There is evidence that rapid growth, caused by too much nitrogenous manure or too high a temperature, is favorable to the disease. Why this should be the case has not been determined. It is probably connected with the manufacture of reserve nitrogen by the cells and its distribution to the rapidly growing parts.

Plants grown under such conditions are less able to stand successfully marked variations in temperature and moister conditions of soil and atmosphere. Variations of this kind favor the development of the disease in the less resistant plants.

Close, clayey soils, packing hard after rains and requiring constant tillage, are not favorable to the even growth of either the tops or roots of tobacco plants. In moist, cloudy weather the plants will grow too fast, and in hot, dry weather the soil is likely to bake, checking growth and making probable injury to the roots in cultivation. Such soils are very favorable to the development of the mosaic disease, as pointed out by Thaxter.[1] He found that loosening the soil by liming and giving partial shade, thus causing a more even condition of growth, very greatly reduced the disease.

Crops grown under cheesecloth covers protected at the side are said to be remarkably free from the disease. The plants make a steady rapid growth, much greater than in ordinary field culture. . . .

The disease is not, so far as observed, produced by a lack of soil nutrients, though from its nature we would expect that a deficiency of nitrogen, phosphoric acid, lime and magnesia might favor its development. Koning[2] says that manuring with kainit and Thomas slag diminishes the extent of the disease. Mayer, Beijerinck and other investigators, however, agree that the trouble is not caused by the lack of any soil nutrients. It appears, so far as my own investigations go, that the trouble cannot be cured by giving the plants additional food of any kind. Overfeeding with nitrogen favors the development of the disease, and there is some evidence that excess of nitrates in the cells may cause an excessive development of the ferments that cause the disease. Very slight attacks of the disease known as "mottled top" are said not to injure the quality of the leaf to a sufficient extent to be noticeable commercially, though they may be less elastic and have a poorer burn and aroma than healthy leaves.

Hunger,[3] in his work on the mosaic of Deli tobacco, verified much of the work of previous investigators, and later, in carefully planned and

[1] Thaxter: Conn. Agr. Exp. Sta. Rept., III, 253 (1899).

[2] Koning, C. J., *loc. cit.*

[3] Hunger, F. W. T.: De Mozaiek-ziekte bij deli Tabak. Med. s'Lands Plantentium, Batavaia. Deel 1: 63 (1903).

executed experiments,[1] proved that the disease was not contagious but was highly infectious. He believed that it could be carried from diseased to healthy leaves simply by touching, especially in the case of the young leaves, a fact that makes it necessary for the workman to use great care when looking for the tobacco bud worms, etc., in the buds. He was of the opinion that a rupture of the leaf was not necessary to induce the mosaic disease in plants.

Selby [2] a year later showed this to be apparently true for tobacco grown in Ohio, and Hunger's statements were in his opinion in all respects confirmed. He also reported that "Blossoms of various plants were inoculated through the nectar by transmission of nectar from diseased plants, as by insect visitation. A slender brush of horse hair was used for this purpose. No evidences of the disease were observed as a result of this method."

Clinton[3] was able to produce the trouble on tomatoes by inoculating with juice from a diseased tobacco plant and from the tomato so infected was able to reproduce the disease on the tobacco again by inoculation from the tomato, again showing the infectious nature of the disease, and that the troubles on the tomato and tobacco were practically identical. This has been repeatedly verified by the writer and many other investigators.

Jensen,[4] in his work on the disease, came to the conclusion that the right way to get at the methods of control of the disease was by experimentation to obtain a resistant strain of tobacco, no matter what the cause of the disease might be, and he carried on some experiments along these lines. As yet no definite results have been reported by the investigators, but the time has probably been too short to obtain results.

Lodewijks[5] stated that by subjecting diseased plants to different colored lights he was able to bring about a cure in some cases. He states: —

> The mosaic disease cannot be diminished or prevented by lessened light intensity. Neither diffused nor colored light has any effect on the disease if the healthy leaves are not able to function in normal daylight. Under the latter condition, however, diffused light exerts a retardation, red light diminishes the trouble, and blue light effects a cure. All the results may then be explained by the hypothesis that the virus formation diminishes with the intensity of the light, while in the healthy leaves, through the action of the virus, an anti-virus is formed, the action of which destroys the virus (immunity and antitoxin formation in the case of animals). . . .
>
> Normally in the metabolism of the tobacco plant a substance is formed, the action of which is opposed to that of the equally normally occurring virus of mosaic disease, perhaps because it binds itself chemically to the latter.

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[1] Hunger, F. W. T.: Die Verbreitung der Mosaikkrankheit infolge der Behandlung des Tabaks. Centralbl. f. Bakt. Par., etc., II: 11: 405–408 (1908).

[2] Selby, A. D.: Tobacco Disease. Ohio Agr. Exp. Sta. Bul. No. 15, 88–95 (1904).

[3] Clinton, G. P.: Notes on Fungous Diseases, etc. Conn. Agr. Exp. Sta. Rept., 1907–08, 857–858.

[4] Jensen, H.: Über die Bekampfung der Mosaikkrankheit der Tabakpflanze. Centralbl. f. Bakt. Par., etc., II: 15: 440–445 (1906).

[5] Lodewijks, T. A., Jr.: Zur Mosaikkrankheit des Tabaks. Rec. Trav. bot. Neerlandais, VII. (1910).

Both substances, virus and anti-virus, may be increased by external factors or conditions. In the first instance the plants become diseased with the mosaic disease; in the latter an immunity against the disease is brought about. Decrease in intensity and cure occur if the virus formation ceases or stops, while at the same time the formation of an anti-virus is taking place normally or is increased.[1]

A discussion of Lodewijks' work is to be found later in this paper.

Allard[2] in a recent work on the disease states that from the results of his experiments he is of the opinion that the trouble is not primarily physiological but is parasitic in nature, but he is unable to throw any light on the nature of the parasite, and in spite of the conclusions drawn by him, none of his results, at least in so far as the writer is able to judge, has in any way weakened the theory that the trouble may be physiological in nature; and some of his results, from the writer's point of view, seem to substantiate this idea of a physiological agency. Two points of great interest are brought out by him, viz., the mosaic as affecting the color of the corolla by blotching, etc., and the carrying of the disease by certain aphids. These points have not been noted before. In the following pages some of his work will be taken up in detail in so far as it seems to bear out or refute work done by the writer.

It may be seen from the foregoing résumé that the theory that the disease is physiological in character has been in the past pretty generally accepted, but the identification of the ultimate causes producing the symptoms varies widely with the different investigators. The writer's conclusions with regard to this point are taken up later in this paper.

Names.

By right of priority the term "mosaic" is the one which should be applied to this disease. It has, however, many local names, and these sometimes are applied differently to the different manifestations of the symptoms; among them may be mentioned the following: "calico," "brindle," "mongrel," "mottle-top," "string leaf," "frenching," etc. Other terms have also been used, but they do not in all cases apply to the "mosaic" alone, hence they are here omitted. The term "infectious chlorosis" as suggested by Clinton is perhaps best descriptive of diseases of this general character, with "mosaic" as a specific type under this division, there being many other infectious, chlorotic diseases of plants quite distinct from the mosaic type.

Description of the Mosaic Disease of Tobacco.

Descriptions of the mosaic disease of tobacco have been repeatedly presented, and the disease itself is so well known that there is little need of repetition at this point, but a brief résumé of the salient characteristics

[1] Translation from abstract of Lodewijks' paper in Bot. Centralbl., 114-518 (1910).

[2] Allard, H. A.: Mosaic Disease of Tobacco. U. S. D. A., n. s., Bur. Plant Ind., Bul. No. 40 (1914).

of the disease will be given so that no misunderstanding may arise, as several other leaf troubles more or less chlorotic in character have often been confounded with the true "mosaic." The disease may show on the leaves at all stages of the growth, from the seedling to the mature plant. It is often difficult in seedlings to diagnose the trouble definitely, as the slight mottling and curl of the leaves may be due to other factors. As a rule, in young plants the leaf is rougher and a *permanent* mottling is observed, very slight in character, however, and not to be confounded with the mottling due to normal metabolic processes which occurs under certain conditions of growth. As the disease progresses, however, the leaf is found to be divided into light and dark green areas; in mild cases there does not appear to be any marked leaf distortion, and the light green areas sometimes verge on the yellow in color. The dark green areas apparently deepen in color with the intensity of the disease, and in extreme cases the leaf is much distorted and the dark portions appear blister-like, due to their more rapid growth. The leaves, as a rule, are much stiffer and thicker to the touch than are the normal healthy leaves. Sometimes in the later stages of the disease there are found dry, dead, brown patches or spots on the leaves, sometimes where the dark green areas were originally, but more often the light green portions show this extreme condition. Both the light and dark areas show abnormalities in structure; nevertheless, the light green areas are the more truly diseased ones, the dark green areas presenting different characteristics, and although showing changes in cell arrangement, etc., function more normally in many respects. Most investigators have held that the light green areas are the diseased portions of a leaf, but some have been of the opinion that the dark green areas are the diseased portions. As will be seen from the writer's experiments the former is the more correct view, as the increase in color intensity and the blistering of the dark green areas is due to the necessarily increased functioning thrown on these portions of the leaf.

Occasionally a leaf may be distorted in such a manner as to present the appearance of being little more than a long filament consisting principally of midrib, with but very little leaf surface. This condition has been observed by the writer in some instances, but should not be confounded with a similar trouble occurring on tobacco in certain regions, which is of an unknown character but which is not the true mosaic as it is not infectious. This latter trouble has been noted particularly in Java, etc., as is reported by Peters[1] in his work on the diseases of tobacco. It has not been observed in tobacco fields in this region by the writer.

It is thought that soil and moisture conditions are responsible at least partially for this disease.

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[1] Peters, L.: Krankheiten und Beschadigung des Tabaks. Mitteil. aus der Kaiser. Anstalt F. Land- u. Forstwirtschaft. Heft, 13: 64 (1912).

Occurrence.

The mosaic disease has been known for years both in Europe and America, and may be said to be present everywhere that tobacco is grown. It apparently is a more serious disease in the tropics and in certain parts of Europe than it is in this country. In New England it has been known for some time, and, although present to a certain extent each year, is not of such great economic importance as in some other localities. In Massachusetts it is found practically everywhere, and some years appears to be much more prevalent over widespread areas than in others. As a rule, however, the disease is not epidemic in character, and often only a comparatively few plants in a field will be found affected.

On certain fields, however, — and these most often are such as have been cropped to tobacco for many years without the practice of cover-cropping or rotation, — mosaic disease is present year after year, and a large percentage of the crop is always badly affected, the plants beginning to show the trouble in from three to four weeks after planting in the field.

The prevalence of the disease in the field, aside from the special cases above noted, is apparently related in some way to conditions in the field during the growing season, or during the time the plants are in the seed bed. There is no question that a large percentage of the infection found in the field, exclusive of that appearing on the sucker growth after topping, or due to infection at the time of transplanting, is due to a primary infection from the seed bed.

While the disease as a rule is first noticed in the field some time after transplanting, very often the seedlings in the beds are affected. This is particularly true in the case of old or carelessly treated beds. It is often very difficult for the casual observer to identify the disease on the seedlings, as the macroscopic or visible symptoms are either very slight or lacking. In this way many plants are transplanted to the field by workmen without their being aware that they are diseased, and the disease becoming more pronounced in the later stages of growth, the infection is laid to the soil in the field, when in reality the infected soil of the seed bed is responsible and not the field soil. As has been stated, the closest examination of the seedlings is necessary to identify the trouble in the seed bed, particularly in mild cases of infection.

From observations made repeatedly, not only on seed beds but also experimentally under controlled conditions in the greenhouse with soils from old beds, afterwards transplanting the seedlings to soil previously not used for tobacco, and using as checks healthy plants from new soil, the writer has come to the conclusion that at least 80 per cent. of our field infections come from the seed bed and *do not* originate in the field as is commonly supposed.

PLATE I.

Mosaic disease on tobacco leaves. (1) Older leaves showing mottling. (2) Leaves showing marked distortion and tendency to string leaf (on right).

PLATE II.

More or less indistinct types of mosaic disease, except (*a*), which is a young leaf with pronounced blisters.

Economic Importance.

It is very difficult to estimate the loss to growers due to mosaic disease, as the prevalence in different localities varies greatly, as also does the intensity of the attack in different seasons. The damage resulting from mosaic disease is twofold: first the plants when severely attacked are smaller and the leaves poorer in quality; secondly, the buyer, if he sees much mosaic in a field, will invariably cut the price a few cents a pound, as the leaves affected do not in many cases make a valuable wrapper and are much poorer in quality. The writer has observed certain fields where the loss would run into hundreds of dollars from this cause alone. The amount of damage done by late mild attacks when the plants are maturing, or appearing on the sucker growth after topping, is practically negligible, and, so far as can be learned, does not in any way injure the commercial leaf. It is always well to clean off the diseased suckers, however, as they present a very ragged appearance, and might injure the sale of the crop to a certain extent. There is no question but that during certain seasons the loss due to mosaic is quite large, but an exact estimate of this loss is difficult to obtain, owing to the many other factors involved.

Infectious Nature of the Disease.

That the mosaic disease is very infectious is well known, and a discussion of the detailed experiments on this point is not necessary. Experimentally it has been repeatedly shown that the juice from all parts of a diseased plant is capable of transmitting the disease, although it should be stated that the percentage of infection obtained from the root extract is considerably lower than that obtained from the leaves. A few of the results obtained are given in the following table, however: —

Table I. — *Infectivity of the Juice from Different Parts of Diseased Plants, August, 1909.*

Part of Diseased Plant used (Plants from Field).	Number of Healthy Seedlings inoculated.	Number of Plants Diseased Three Weeks after Inoculation.
Leaves showing disease,	10 (juice; needle pricks),	10
Control,	10 (distilled water; needle pricks),	-
Leaves showing disease,	10 (insertion of tissue into veins),	9
Control,	6 (insertion of healthy tissue into veins),	-
Basal leaves (not showing disease),	12 (juice; needle pricks),	10
Control,	5 (distilled water; needle pricks),	1
Roots,	21 (juice; needle pricks),	14
Control,	7 (distilled water; needle pricks),	-
Roots,	16 (insertion of tissue into veins),	6

Later experiments with the roots of other diseased plants gave similar low results.

It is a very easy matter to infect seedlings at the time of transplanting, and the writer has repeatedly seen many cases in the field which could only have been brought about by such infection. It is only necessary to get some of the juice from the diseased plant on to the hands to transmit the disease by handling healthy plants, the causal agent gaining entrance through the broken ends of roots, leaf hairs or broken and abraded leaf areas. In some of the experiments conducted relative to this point, a very high percentage of infection has been obtained. In one case where the juice from a diseased plant was very thoroughly rubbed on the hands, and 40 healthy seedlings immediately set, no care being used to guard against bruising the leaves, etc., 31 plants developed the disease in two weeks' time. In another experiment where 62 seedlings were subjected to the same treatment, 30 plants developed the disease; in still another, series of 28 seedlings, 21 developed the disease. Controls planted at the same time, handled with a hand rubbed with the juice of a healthy leaf developed the mosaic in only a few isolated cases. From the above it can easily be seen that great care should be exercised in the matter of handling the seedlings, especially diseased seedlings.

Contagious Nature of the Disease.

In spite of the fact that it is held by some investigators that the mosaic disease is contagious, the writer has never been able to satisfactorily demonstrate that it is. Under carefully controlled conditions in the greenhouse, guarding against accidental infection, it has been impossible to demonstrate the contagious nature of the disease. In isolated instances, indeed, apparent contagion has occurred, but it is believed that these cases were due to accidental infection, as the percentage was so low, — less than 2 per cent., — and under the conditions the plants were subjected to, such as contact, spraying of the juice on leaves, etc., the percentage should have been much higher if contagion was to be held responsible.

It is a fact that it is only necessary to break or rupture the trichomes or hairs on the leaf, subsequently spraying with diseased juice, to obtain infection, although this method does not give a very high percentage. It can easily be seen that such a rupture may be very easily brought about, and hence apparent contagion occur. As is stated elsewhere in this paper, insect and other carriers may also play a part in these so-called cases of contagion.

Pathological Anatomy.

Leaves.

As might be supposed, there are great differences in structure between normal, healthy leaves and leaves affected with the mosaic disease. These differences are greatest, naturally, in badly diseased leaves. Woods [1] was one of the first to point out this fact, and his statements have been repeatedly verified by the writer. He stated that the light colored areas were not normal, and that "this difference consists in the fact that in badly diseased plants the palisade parenchyma of the light colored areas is not developed at all. All the tissue between the upper and lower epidermis consists of a spongy or respiratory parenchyma rather more closely packed than normal. In moderately diseased plants the palisade parenchyma of the light area is greatly modified. Normally the palisade parenchyma cells of a healthy plant are from four to six times as long as broad. In a moderately diseased plant, however, the cells are nearly as broad as they are long, or at most, not more than twice as long as broad. As a rule, the modified cells of the leaf pass abruptly into the normal cells of the green area."

From the above it can be seen that Woods was of the opinion that the light green areas were abnormal or diseased, and that the dark green areas were normal and healthy. The writer in his observations found this to be true in general, but occasionally the dark green areas showed a more closely packed parenchyma than in normal leaves, and *always the palisade layer was well developed* and approached the normal in character. The development or non-development of the palisade layer, as Woods hinted, is dependent on the degree of severity of the disease. The lighter the attack the less are the palisade cells and parenchyma tissue altered, and *vice-versa*. This the writer found to be true in so far as anatomical differences were concerned, but as will be noted later, the dark green, apparently normal, healthy tissue contained some of the infective agent of the disease.

The structure of the dark green areas varies only slightly from that of the normal leaf, with the few exceptions above noted, and may be considered normal in character. The writer has sectioned many leaves in all stages of disease, and these structural differences have always been found to occur in the manner above indicated. These differences in structure have been taken up more or less in detail, as some investigators have held, and still hold, that the dark green areas are the part diseased, and that the light green areas are normal, inasmuch as they approach the normal leaf in color in many cases, most probably basing their assumption on the fact that the dark areas form blister-like growths and are sometimes darker in color than normal leaves. No one recently appears to have

[1] Woods, A. F.: Inhibiting Action of Oxidase on Diastase. Science, n. s., XI., No. 262, 17–19 (1900).

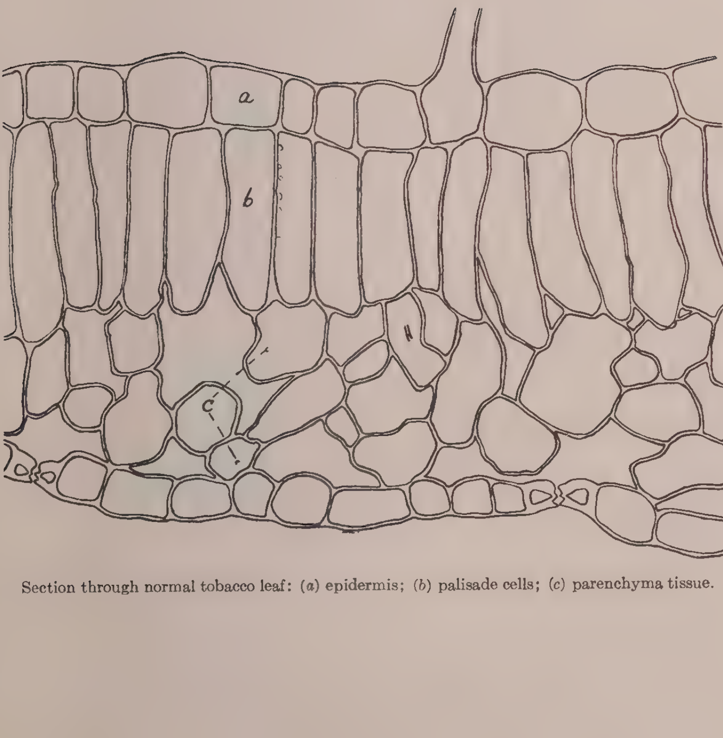
investigated the structure of the dark and light areas carefully in the case of the tobacco, except Woods. It was to verify Woods' statements that the writer took up this phase of the matter, and mention will again be made of it in connection with the biochemistry of the leaf. There can be no doubt as to the correctness of Woods' contention that the light green areas are abnormal and diseased; but that the dark green areas are not diseased, at least in certain cases, cannot be so definitely stated. Their structure may be somewhat modified by the increased functioning thrown on the healthy cells. On the other hand, it is fallacious to state that the light green are the healthy, and the dark green are the diseased, portions of a leaf.

Plates III. and IV. show three cross sections from leaves, III. showing the cross section of a healthy leaf; IV., that of the light green area of a diseased leaf and of a dark green area of the same leaf. It will be noted that the palisade layer is practically suppressed in IV. (1), or the light green portion, while in IV. (2) the palisade layer approaches the normal in character except for a closer packing of cells in general. Milder cases of diseased leaves vary between these limits. These figures are from *camera lucida* drawings of material killed and fixed in medium chrom-acetic acid. In the material used the normal leaf section is somewhat thicker than those of the diseased leaf, but for comparative purposes is perfectly satisfactory.

Stems.

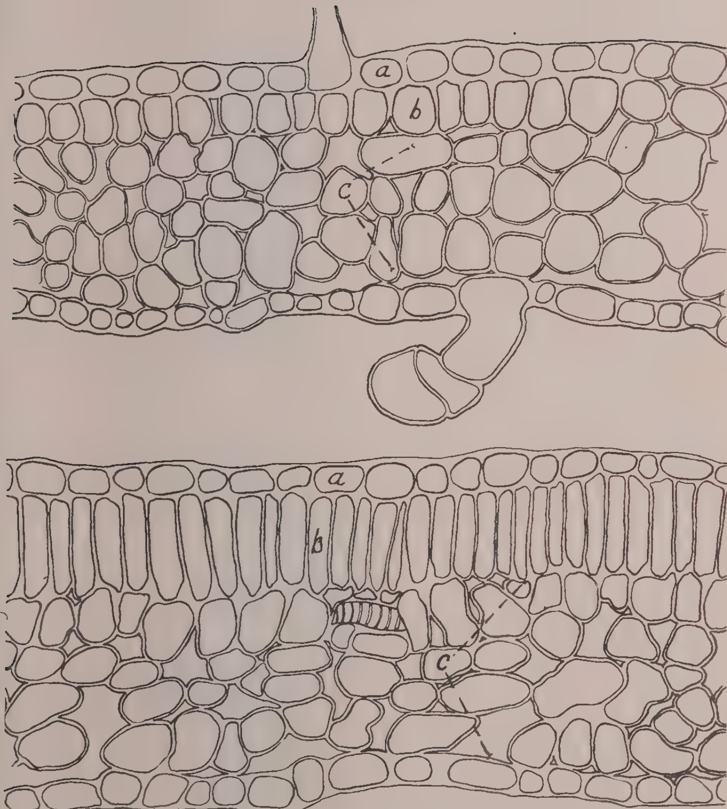
The anatomical differences in the leaves of healthy and diseased tobacco plants have been given in the preceding paragraphs, and as it was desired to carry the investigations further to cover the entire plant, repeated examinations were made of both cross sections and longi-sections of stems of plants in various stages of disease, and also of healthy, normal plants grown both in the field and greenhouse. It should be stated at this point that occasionally the writer has observed on the stems of some badly mosaicked plants a mottling, or, rather, a streaking of the stem, a portion of which would be darker green than the remainder, and this is without question a manifestation of the mosaic disease. Sections of such stems, however, showed absolutely no variation in structure from those of normal plants, and in no case, although the examinations covered an extended period of time, was it possible to show any structural difference between the stems of badly diseased mosaic plants and those of healthy plants of the same age. Examinations of the stem close to the terminal apex of the plant revealed the same conditions as those of other parts of the stem. No differences were observable except in the matter of size and arrangement of cells, such as would naturally be expected when we take into consideration the differences in size and development of the stem near the terminal apex and progressively towards the base.

PLATE III.



Section through normal tobacco leaf: (*a*) epidermis; (*b*) palisade cells; (*c*) parenchyma tissue.

PLATE IV.



2.

Sections through mosaic-diseased leaves. (1) Light green area: (*a*) epidermis; (*b*) palisade cells; (*c*) parenchyma tissue. (2) Dark green area: (*a*) epidermis; (*b*) palisade cells; (*c*) parenchyma tissue.

Roots.

In the same manner roots of mosaicked and healthy plants were examined at various times under all conditions of growth and severity of disease, and in every case the root structure was found to be normal. Root tips from healthy and diseased plants showed absolutely no differences in structure. It might be anticipated that, as the disease first manifests itself in the dividing cells of the leaves, there might be a supplementary differentiation, so to speak, of the meristematic tissue at the growing point of the root, functioning co-ordinately with that of the aerial part of the plant. No such condition was observable, however, and, so far as the writer has been able to find, there is no manifestation of local cell disturbances in the root such as are found in the leaf tissue.

The causal agent of the disease, however, as has previously been noted, is without question present *in all parts of the plant*, and it should not be stated that it is confined to those parts which show structural variation.

Fungi and the Mosaic Disease.

Almost from the first it has been established that no fungi are associated with the cause and development of the mosaic disease of tobacco. In no case where careful work has been conducted under conditions eliminating the possibility of accidental infection has any fungus been found associated with the trouble. Cultures of fungi obtained occasionally from leaves have always been traceable to careless manipulation or external infection, and the fungus obtained failed to infect healthy plants, no matter what methods of inoculation were used.

The writer has occasionally obtained cultures on various media such as oat agar, tobacco leaf agar and prune agar, from the tissue of the so-called "rusted" spots which are sometimes a late manifestation of the last stages of the mosaic; but, as with previous investigators, it was found impossible to infect healthy plants from these cultures, either by needle pricks, spraying, or inserting the fungus into incisions in the leaf or stem.

These experiments with fungi were made merely to demonstrate to the writer's own satisfaction that they could not be the causative agents of the disease, as there might be a possibility that they were latent in the plant during the earlier stages of the disease and only developed superficially during the later stages.

According to Jenkins [1] and others these rusted spots which are sometimes observed are primarily caused by a drying out and disintegration of the cell tissue, which has been weakened by the disease and which thus forms a suitable medium, under favorable conditions, for the development of secondary fungi and micro-organisms. This view is also held by the writer as a result of observations extending over a series of years.

[1] Jenkins, E. H.: Studies on the Tobacco Crop of Connecticut. Conn. Agr. Exp. Sta. Bul. No. 180, p. 56 (1914).

Bacteria and the Mosaic Disease.

Among the many theories advanced regarding the cause of the mosaic the chief one for some time, particularly among the earlier investigators, was that of bacterial infection either through the agency of infected soil or otherwise. Mayer,[1] in his rather extended study of the disease, came to the conclusion that it was caused by bacteria, but was unable to isolate the organism. Prilleux and Delacroix[2] claimed to have found an organism associated with the mosaicked leaves, but their descriptions leave one in doubt as to whether they were working with the true mosaic disease or not. It is very probable that they were dealing with another disease which occurs in France, but which is somewhat different from the mosaic disease. The next important work on the bacteria supposedly connected with this disease was done by Iwanowski.[3] He isolated several organisms from the juice of diseased leaves, and by reinoculation was able to cause infection, but only in a very small number of instances. This he explains by a probable attenuation of the organism when grown on artificial media. Hunger,[4] in a very critical review of the bacterial theory, stated that he was unable in any way to substantiate the findings of Iwanowski, and that although he observed certain bodies in the cells, he was not able to classify them as either bacteria or plasmodia, as they disappeared after heating with phenol chloral hydrate, while the rest of the cell contents were unaffected. More recently Allard[5] has advanced the opinion as a result of his investigations that the disease is parasitic in nature but does not attempt to discuss the character of the parasite, and apparently has made little attempt to demonstrate anatomically the presence or absence of bacteria. Hunger's work is probably the most satisfactory of its kind along this line.

The writer has made examinations of diseased plants, sectioning leaves, stems and even the roots, but has never been able satisfactorily to demonstrate the presence of bacteria in the tissues. In this work a variety of stains were used, chief of which, however, were Ziehl's carbol fuchsin and Heidenhain's iron hæmotoxylin.

It is to be noted in this connection that all investigators have apparently confined their studies to the leaves or part of the plant in which the disease showed itself, and very few attempts, if any, have been made to study the question of the possible presence of bacteria in tissue far removed from the diseased portions. In view of the fact that the juice from all

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[1] Mayer, A.: Over de in Nederland dikwijk voorkomende Mozaikziekte der Tabak. Land. Tijdschr. (1885).

[2] Prilleux, E. E. and Delacroix, G.: Maladies bacillaires de divers végétaux. Compt. Rend. Acad. Sci. Paris, 118: 668–671 (1894).

[3] Iwanowski, D.: Über die Mosaikkrankheit der Tabakspflanze, Zeit. f. Pflanzenkrank, 13: 1–41, pl. 1–3 (1903).

[4] Hunger, F. W. T.: Untersuchungen und Betrachtungen uber die Mosaikkrankheit der Tabakspflanze. Zeit. f. Pflanzenkrank, 15: 257–311 (1905).

[5] Allard, H. A.: Mosaic Disease of Tobacco. U. S. D. A., Bur. Plant Ind. Bul. No. 40 (1914).

parts of a diseased plant will cause infection, it would be natural to suppose that if bacteria were the causal agent, it should be possible to demonstrate their presence in the different parts of a diseased plant. This has never been done, and in the writer's study of the anatomy of diseased plants it has never been possible to demonstrate the presence of bacteria in the different tissues. The writer has many times attempted to obtain cultures of bacteria from diseased tissue, and in some cases cultures of organisms were obtained on various media, but they proved in every case to be secondary in character, and were not capable of reproducing the disease. In the light of all later investigations the evidence points overwhelmingly to the absence of bacteria, in the present-day sense of the term, as the causal agent of the disease.

Dissemination Agents.

Insects.

The fact that many fungous and bacterial diseases are often transmitted by insects, as well as other agents, has been long known and thoroughly established, but until Allard (*loc. cit.*) called attention to the fact that the mosaic disease could be carried by aphids, and one in particular (*Macrosiphum tobaci* Perg.), nothing had been published on this phase of the matter. Allard in well-controlled experiments demonstrated beyond a reasonable doubt that the disease was so communicated. Clinton (*loc. cit.*) made a few observations on the infection of healthy plants by the tobacco horn worms which had been feeding on diseased leaves, but was unable to demonstrate that the disease could be so transmitted either by the excreta ejected by the worm or by its biting and feeding on the healthy plants. His results were negative in the few experiments made. Observations made in the field during the progress of the writer's work have not shown conclusively that the disease is communicated by biting insects, such as the tobacco horn worm, grasshoppers and a small black flea beetle of more or less common occurrence in our fields.

Occasionally aphids have been found infesting the leaves of tobacco in our fields, but so far as could be judged were present in too small numbers to be active agents in transmitting the trouble. As a rule, comparatively few aphid infestations are found in our tobacco fields.

In the greenhouse during several winters tobacco plants grown in benches were infested with white fly, and it was at first feared that they might carry the infection from diseased to healthy plants in the same benches. This, however, was not the case, and it has never been possible to demonstrate positively that the white fly is an active agent in the spread of the disease. This insect is, of course, of rare occurrence in our fields, but may possibly do damage in the south. It apparently feeds and breeds freely under greenhouse conditions on the underside of the leaves.

In order to ascertain more definitely the possibility of infection by these insects, adult white flies from badly mosaicked leaves were carefully re-

moved and placed on the underside of the leaves of tobacco plants, enclosed in a small cloth-covered cage, and were allowed to remain on the tobacco leaves of the plants in these cages for four days. After this length of time the plants were removed from the cages and placed on a bench at some distance from those containing mosaicked plants badly infested with white fly. On none of the plants did mosaic develop. The plants were later placed in close juxtaposition to those in the original benches, which, as indicated, were at this time heavily infested with the white fly and badly mosaicked, but although the plants remained until maturity, no cases of mosaic developed on them in spite of a heavy infestation of white fly.

The writer's observations on the activities of aphids as carriers of infection have not been so extensive as in the case of the white fly, as only minor infestations of the former occurred in the greenhouses; and the indications pointed to the fact that, although there were a certain number of aphids present on the leaves of both healthy and diseased plants, so far as was observable no cases of infection from this source arose, as the mosaic developed only on an average of 1 case out of 30, except on the plants which were artificially inoculated with the juice from diseased leaves. It should be stated, however, that aphids present in the greenhouse were not of the same species as that under consideration by Allard, and there is no reason to doubt the accuracy of his observations on the species *tabaci* Perg.

The question of insects as carriers of the mosaic disease as well as of many other diseases is still open to discussion; and it may be that in the case of the mosaic a very heavy infestation of aphids is necessary to bring about a successful infection of healthy plants, as the amount of active infective material carried by such insects would in any case be very small, and accumulative effects of the activities of several insects might be necessary to introduce a sufficient amount of the active principle to transmit the disease.

Workmen.

It has been shown that the disease is highly infectious and it has also been proved repeatedly by many investigators that it is very easy to transmit the disease to healthy plants at the time of transplanting. A workman handling diseased seedlings, and subsequently healthy ones, will very often infect them. Several instances of this have come to the writer's attention, every other plant for some distance in a row developing mosaic within a month after transplanting. The same condition has also been observed by Clinton (*loc. cit.*) in Connecticut, and can only be explained by the fact that the workman's hands were infected through handling a diseased plant, and the infection then transmitted to healthy ones, the causal agent being introduced through broken tissue of the leaves or roots of the seedlings. This method of transmission is particularly striking in the above case, as the same individual plants every other plant in a row when working the ordinary planter. Of course, there

have been many cases where every plant for some distance in a row has developed mosaic, but this might be explained if it is assumed that *both* workmen had handled diseased seedlings, or if a number of plants in the lot were diseased. In time, the causal agent becomes so attenuated that infection ceases, and the remainder of the row remains healthy. Experimentally, this method of transmission has also been shown to be possible, and a high percentage of infection has been obtained. In one experiment, after thoroughly rubbing the hands with the tissue of a diseased plant, and then pulling and transplanting healthy seedlings, over 80 per cent. of the transplants became mosaicked within a month. Only a relatively small number of seedlings in this instance were treated in this way, however, the total being 28, of which 24 developed mosaic symptoms within three weeks.

Another manner of transmission is by cultivation. If some of the sap from a diseased plant comes in contact with the tools, etc., employed, there is a possibility that the infection might be carried to healthy plants by this means, but the percentage of infection of this character is probably very low in actual field practice.

The workmen when budding and topping are very often carriers of infection, as they are not as a rule careful to leave untouched the plants showing mosaic symptoms but take the plants as they come, and thus spread the disease to many healthy plants. This method of dissemination has been very often observed, and perhaps is the most fruitful source of infection in the field. The subsequent new growth will almost invariably be mosaic in character, as will also the suckers developing later. The amount of damage to the marketable leaves, however, providing the suckers are removed, is very slight, if any, and cannot be said to injure the leaf in any way, at least in so far as our observations bear on this point. If the suckers are left, however, the plants present a ragged appearance, and the mosaic on the suckers is quite noticeable, and might injure the sale of the crop at the price it ought to command.

Seed.

The causal agent is *not* carried by the seed, and seed from mosaic plants has never produced a larger percentage of mosaicked seedlings than seed collected from healthy plants, when germinated and grown under the same conditions. It is difficult to conceive of this, as it has been shown by Allard (*loc. cit.*) that the tissues closely enveloping the seed in the pod are capable of causing infection; but the writer has saved seed from badly mosaicked plants for three successive years, and the seedlings from such seed showed no signs of the disease, unless infection was produced artificially through some external agency.

It should be pointed out, however, that there is the possibility that the vigor of the seed from mosaicked plants may be less than that from healthy ones, and consequently the plants developed from such seeds, being weaker, might be more susceptible to the factors active in the production of

mosaic symptoms. It is impossible to make a definite statement on this point, however, as the writer has not been able to gather sufficient data over a series of years to prove or disprove it.

Fertilization in Relation to Mosaic Disease.

It has been repeatedly shown by many investigators (see historical summary) that a lack of plant food alone will not suffice to produce the mosaic disease, and the writer has also, in connection with the tomato, shown that an *excess* of nitrogen, potash, phosphoric acid and lime will not produce nor intensify the disease.[1]

The same has been found to be true for tobacco. In our experiments on tobacco, the method made use of was to add to each pot the proper amount of a complete tobacco fertilizer (in this case applied at the rate of 3,000 pounds per acre), and then to add an additional amount of nitrogen, potash and phosphoric acid in quickly available forms, equal to that already present. No mosaic was produced in any case, although where the amount of nitrogen was trebled a rather peculiar malformation of the leaves was observed which at first sight might have been mistaken for mosaic symptoms. All inoculations failed to take, however, and the trouble therefore could not have been the true mosaic.

It has been held that liming would lessen the prevalence of the disease, but the writer's observations and experiments do not bear out this statement. Under field conditions this may be the case in certain seasons, but continued observations from year to year on heavily limed areas show no appreciable lessening of the number of mosaicked plants. Seedlings and plants grown in the greenhouse in soil known to be heavily infected indicated the same results, as also did the work on new soil with mosaicked seedlings. Here lime was applied in varying amounts at the rate of from 500 to 6,000 pounds per acre. No appreciable effect on the mosaic disease was observable. The results obtained are given in the following tables: —

Table II. — *Effect of Liming on Mosaic.*

[New soil, lime, mosaicked seedlings.]

Lime (Pounds per Acre).	New Soil in Pots (Number planted with Mosaicked Seedlings).	Number of Plants showing Recovery One Month after Planting.
500,	40	–
1,000,	28	–
2,000,	34	–
4,000,	12	–
6,000,	10	–
No lime (check),	5	–

[1] Twentieth Annual Report, Mass. Agr. Exp. Sta. (1908), p. 140.

The lime was applied to this new soil, in the different amounts indicated, one week previous to the setting of the plants.

No appreciable differences were observable in the subsequent growth as regards intensity of mosaic symptoms, all the plants being comparatively evenly mosaicked. There was not a single case of recovery.

TABLE III. — *Effect of Liming on Mosaic.*

[Infected seed bed soil, lime, seed.]

LIME (POUNDS PER ACRE).	Per Cent Infection (Seedlings Twelve Weeks Old).
500,	12.0
1,000,	18.4
2,000,	9.8
4,000,	21.0
6,000,	8.6
No lime (check),	13.7

The lime was here applied to a soil which was heavily infected, and the seed sowed very thinly in the flats containing the various amounts of lime and soil. The seedlings were allowed to grow in the flats until they were counted. They were naturally crowded somewhat, but were free from insects during the period of growth. It is possible that some infection may have occurred, however, but there are very strong indications that liming had no beneficial action in lessening the disease. As the results are so variable the matter cannot be considered as absolutely settled, but certainly no consistently favorable results were obtained in this experiment from the use of lime.

EFFECT OF COLORED LIGHT ON MOSAIC DISEASE.

In connection with work on the mosaic disease of tobacco it had long been noted, in that section of the Connecticut Valley where the crop was grown under shade, that the plants appeared in general to be much less affected with the mosaic disease than were those grown in the open. This fact has already been noted by Sturgis [1] in Connecticut. Investigations were outlined, in conjunction with other work on this disease already under way, relative to a study of the effects of various light conditions on the intensification or reduction of the disease. While the writer's preliminary work was in progress, Lodewijks [2] published a paper

[1] Sturgis, W. C.: On the Effects, on Tobacco, of Shading and the Application of Lime. Conn. Agr. Exp. Sta. Ann. Rept., 23, 252–261 (1899).

[2] Lodewijks, J. A., Jr.: Zur Mosaikkrankheit des Tabaks. Rec. Trav. Neerlandais, Vol. 7, 107–129 (1910).

on the effects of colored light on mosaic-diseased plants, and as a result of his experiments stated that a cure was effected by blue light, red light diminished the disease, and suffused light checked it somewhat. In brief, his methods of experimentation and conclusion were as follows: —

The diseased leaves of a plant were covered with a cloth hood of the desired color, of a sufficient size to allow ample room for growth. The apparently healthy basal leaves were left uncovered and exposed to the normal daylight. After a time the hoods were removed, and it was found that in the case of the plants exposed under the blue hood a cure was effected; those exposed under a red hood showed a diminution in the severity of the disease; and in the case of plants exposed to the suffused light alone the disease was somewhat checked. The cloth used for the red and blue hoods was a rather coarse cotton material similar to that used for making flags.

Several investigators had noted the apparent beneficial effects resulting from growing diseased plants in suffused light, but Lodewijks was the first to really study the effects produced by colored light, although Bauer appears to have made some observations on this point. As in no case could the writer find that Lodewijks in his work had reinoculated from the apparently cured plants to healthy ones, to prove the presence or absence of the causal agent, and as it is often present and active in apparently healthy leaves of diseased plants, as has been shown many times, it was thought necessary to settle the point as to the presence or absence of the causal agent in plants treated as in Lodewijks' work.

Method. — The method of treatment of diseased plants was in every way similar to that employed by Lodewijks as to texture of cloth, methods of covering the plants, etc. The cloth covers were held away from the plant by means of wire hoops, and the cloth was tied around the stem of the plant below the diseased leaves. Plate V. shows a hood in place over a field-grown plant, and gives a clear idea of the arrangement of the hoops, etc.

The cloth used was a coarse grade of cotton, and the colors were cadmium orange, ox-blood red and indulin blue.[1]

Plants showing well-developed symptoms of the mosaic disease were selected for the experiment, none of which had less than four characteristically diseased leaves, the lower remaining leaves apparently healthy. The hoods were placed over the diseased leaves as above noted, and left on for the required time, in most of the experiments twenty to thirty days. At the end of this period the hoods were removed and the plants carefully examined for *visible* symptoms of the disease. Two leaves from the upper (*i.e.*, the part under the hood) portion of the plant were removed under absolutely aseptic conditions, the juice expressed and healthy plants inoculated therewith by means of glass capillaries inserted just below the terminal leaflets. Control inoculations with distilled water and boiled juice were also made at the same time. The plants, after the

[1] Ridgway, Robert: Color Standards and Color Nomenclature. Washington, D. C. (1912).

Effect of colored light on mosaic disease; showing method of attaching hoods over leaves.

removal of the leaves above mentioned, were allowed to grow to maturity under normal light conditions.

Most of the experiments were carried on in the greenhouse, where temperature and other conditions were under more direct control than in the field, although field experiments later repeated gave the same results, but, of course, in this case there was a greater chance of subsequent infection through careless handling, insect attacks, etc. In the following paragraphs are tabulated the results of a typical series of experiments relative to the effects of light on mosaicked plants.

Experimental Data.

Red Cloth. — Three plants were covered with the red cloth hoods for twenty days. The covers were then removed, and in all cases visible symptoms of the disease were still present, although the color variation between light and dark green areas was not so marked as at the beginning of the experiment. All the new growth, in addition to the leaves diseased at the time the hoods were put on, also showed the mottling distinctly. A week after the hoods were removed all the plants still showed the disease in undiminished severity.

Healthy plants inoculated with the juice from the leaves confined under the hood became diseased in from nine to eighteen days' time. Controls inoculated in the same manner with boiled juice from the same leaves, and with distilled sterile water, remained with very few exceptions healthy. Table IV. gives the results of the inoculation experiments in one series.

TABLE IV. — *Result of Inoculation with Juice from Plants grown under Red Hoods.*

PLANT No.	Number of Healthy Plants Inoculated with Juice from Leaves of Treated Plant.	Number of Inoculated Plants showing Mosaic at the End of Eighteen Days.
A-1,	6	6
B-1,	7	6
C-1,	4	4

Controls inoculated with boiled juice, 10; diseased in eighteen days, 1.
Controls inoculated with distilled, sterile water, 10; diseased in eighteen days, 0.

From the above results it may be seen that there was a diminution in the color variation in diseased leaves; it was not of a permanent character, the plants all showing the disease in undiminished severity again after a short exposure to normal daylight. The causal agent of the disease was still highly infectious.

In a second series the hoods were allowed to remain over the plants for thirty days, as it was thought that a twenty-day exposure might have been too short, but no appreciable variation in the results was obtained as a result of the longer treatment.

Orange Cloth. — In this series two plants were covered with orange hoods for a period of thirty days. On removing the covers it was found that the visible symptoms of the disease were, if anything, intensified. The growth was somewhat more spindling, the leaves narrower, and the light and dark green areas very clearly defined. Infection was produced from both plants by inoculation into healthy plants. The causal agent was very active and highly infectious.

Blue Cloth. — The diseased parts of three plants were covered with blue cloth hoods, as in the preceding experiments, for a period of twenty-five days. The covers were then removed and a careful examination of the leaves made. On plants A-2 and B-2 no visible symptoms of the mosaic disease could be observed, although a slight tendency towards curling was noticeable on a few of the leaves. The leaves were all uniformly light green in color, and aside from this, appeared normal. Plant C-2, however, showed on two leaves a slight mottling. Two weeks after the hoods were removed, plants A-2 and B-2 did not show any marked symptoms of the mosaic disease other than a faint mottling of a few leaves, not sufficient, however, to seriously injure the leaf. Plant C-2 developed mosaic again in the same length of time, but not as seriously as before the treatment. It may be that the mottling on A-2 and B-2 was due to the maturing of the plant, although this mottling is usually distinctive enough to be readily differentiated from that caused by the mosaic disease.

Healthy plants inoculated with the juice of leaves from plants A-2, B-2 and C-2 contracted the disease almost without exception. Controls inoculated with boiled juice failed to develop the disease. Table V gives the results of the inoculations.

TABLE V. — *Results of Inoculations with Juice from Plants grown under Blue Hoods.*

PLANT NO.	Number of Healthy Plants Inoculated with Juice from Leaves of Treated Plant.	Number of Inoculated Plants showing Mosaic at End of Eighteen Days.
A-2,	8	5
B-2,	4	4
C-2,	10	9

Controls inoculated with boiled juice, 6; diseased in eighteen days, 0.
Controls inoculated with sterile distilled water, 6; diseased in eighteen days, 1.

The above results show that when blue light is used there is a suppression of leaf color variation more or less permanent in character, the treated plants, with one exception, showing no typical symptoms of the disease for at least two weeks subsequent to the removal of the hoods. It cannot be said, however, that the disease was controlled, as inoculation of healthy plants with the juice from these leaves produced the disease in nearly every case.

The causal agent of the disease was still very active in the *apparently normal fully recovered leaves*, and was highly infectious.

Discussion of Results. — The results of these experiments do not agree entirely with those obtained by Lodewijks, particularly in the case of action of the blue light, inasmuch as the plants covered with the blue hoods, although showing an *apparent* recovery from the mosaic, still contained the causal agent of the disease, and by inoculation with the juice expressed from these plants into healthy plants the disease was again produced in practically all cases. It should be noted that the visible symptoms of the disease were suppressed, the reason for which may be as Allard (*loc. cit.*) suggests in his work on the mosaic disease of tobacco. He states, with respect to Lodewijks' observations, "If the malady in question was true infectious mosaic disease, one is inclined to believe that covering the young plants temporarily reduced the color contrasts of the mottled areas. These changes may have led Lodewijks to conclude that a partial or a complete cure had been effected in his experiments."

It might be inferred from the above that on the removal of the hoods exposing the plants to normal daylight, they would soon regain the color contrast, but this is not entirely so in the case of the blue light, as has been shown. The apparent recovery, therefore, is not entirely the result of a suppression of color contrast due to the action of blue light on the leaves as suggested by Allard, but is undoubtedly so in part.

It is evident that the treatment of plants as above recorded does not destroy the causal agent of the mosaic disease, whatever may be its character, the treated leaves apparently still containing the causal agent, very probably in the same manner as do the parts of a plant which do not show visible symptoms of the disease, as the stem, lower leaves, roots, etc., the juice of which is often highly infectious. It would appear from the results that the new terminal growth subsequent to the removal of the hoods would develop the trouble, and this was the case in plant C-2, but not apparently so with plants A-2 and B-2. Lodewijks' opinion, therefore, that in the plant a "virus" and "anti-virus" are present, and that certain abnormal conditions cause the "virus" to be produced in excess, bringing about a mosaicked appearance, while if the "anti-virus" is produced in excess, immunity is secured, will hardly hold, as it is clearly shown that even after apparent cure the causal agent is present and active.

It is significant to note that under the influence of blue light both assimilation and starch formation are decreased, thus bringing about a

partial starvation, as it were, not, however, serious enough to reduce greatly the total starch formation and assimilation of the whole plant; while at the same time the chlorophyll production is very little changed if a comparison of the color of the normal and treated leaves can be taken as a basis of such a comparison. This latter fact has already been noted by Lodewijks in his work on the disease.

It is, therefore, indicated by the results obtained in the preceding experiments that the different colors have little or no effect on the causal agent of the disease, but in the case of the blue there is a strong depression of the macroscopic symptoms of the disease.

Biochemical Studies.

Enzyme Activities in Healthy and Diseased Plants.

The study of enzymes in relation to diseases, particularly those of a so-called physiological nature has not been extensively gone into as yet by investigators, but it is believed that a study of their activities and reactions should be made, not only in the case of physiological troubles, but also those caused by fungi and bacteria, as it is the writer's firm belief that the activities of a large number of the fungi, and their effects on the respective hosts, are in a great measure due to the action of either exoenzymes or endoenzymes produced by the fungi concerned. There is a possibility that the future may show a great advance in the study of host resistance, etc., when the conditions under which enzyme activity in fungi and bacteria takes place are better known, and plants may possibly be bred to a condition of producing either a sap in which these activities cannot take place, or will produce anti-enzymes which will inhibit the activities of the enzymes contained in the respective fungi.

Although many have made a study of this disease, very few have concerned themselves with the question of the enzyme activities; among the first to make mention of this phase of the question was Woods (*loc. cit.*), who found that the enzymes designated as peroxidases were at least diffusable, and occurred apparently in larger amount in diseased leaves than in healthy ones; also that their action was twice as strong in the light green areas as in the darker portions of the leaf. Koning (*loc. cit.*), as a result of his investigations, came to the conclusion that the disease was caused by a certain enzyme, which he stated to be oxidase, and the action of which he described. He believed that it was formed in the plant under certain conditions. Heintzel[1] also found oxidizing enzymes present which were more active, if not present in greater amounts, in diseased plants than in the normal plants. Woods later (1902), in his work on the mosaic disease, verified his former observation, and stated further that the diastase activity was much inhibited in the case of diseased plants. He attributed the lessened diastase activity to the presence of excessive

[1] Heintzel, K.: Contagiose Pflanzenkrankheiten ohne Microben mit besonderer Berücksichtigung der Mosaikkrankheit der Tabaksblätter. Erlangen, 46 p., 1 pl. (Inaugural Dissertation) (1900).

amounts of oxidizing enzymes, and showed experimentally that diastatic action is inhibited by the presence of oxidizing enzymes. This is the only work that has been accomplished up to the present time, so far as relates to a study of the enzyme activities involved in this disease. Only two enzymes have been considered, namely, the oxidase and diastase, and it should be stated that in the light of later developments in the determination and estimation of enzyme preparations and activities the results obtained in some cases might well be open to some criticism.

Loew,[1] while working with tobacco, discovered the presence of an enzyme which he called catalase, but he made no observations relative to its activities in the case of mosaic-diseased plants. The results of the writer's studies on enzyme activities of healthy and mosaic plants are detailed below.

Method. — In the experiments here detailed the enzymes under discussion were studied, in so far as was possible, (1) with regard to their presence or absence in (*a*) leaves, (*b*) stems and (*c*) roots of healthy and diseased plants (this was considered necessary, as it has been found that, irrespective of the parts showing visible symptoms of the disease, the sap from all other parts also is capable of transmitting the trouble); (2) with regard to the age of the plant; (3) with regard to the growth of the plant under different conditions. These will be discussed in detail under their respective sections.

The methods employed for the estimation were for the most part those which by experience have been found satisfactory, and in the main give quantitative results; in some cases the results are more or less qualitative in nature, owing to our present insufficient knowledge of the methods of isolation and action of the enzyme involved.

It should be stated that plants used in the experiments were both field and greenhouse grown, but no essential differences in results were obtained from the two series. The individual experiments will not be given in detail, but as the determinations of any given series were made in every case in the same manner, only average results with the maximum and minimum readings will be given. The experiments are, however, described in sufficient detail to enable those interested to follow the methods employed closely enough to check up the work of the writer.

Catalase (*leaves*). — A comparison was made of the catalase activity of healthy and diseased leaves, as it had been noted as early as 1908 by the writer that there was apparently a great difference between the catalase activity of healthy and mosaic-diseased tomato leaves, and later the same was found to be true in the case of tobacco. At that time only rough determinations were made, but since then the writer has made hundreds of determinations, the results of which have borne out the observations made then, and indisputably established the fact that there is a wide difference in the catalase activity of healthy and diseased leaves.

[1] Loew, O.: Catalase: A New Enzyme of General Occurrence, with Special Reference to the Tobacco Plant. U. S. D. A., Bur. Plant Ind., Bul. No. 68 (1901).

In all the experiments freshly collected material was used, and the determinations made almost immediately after collection. The usual procedure was as follows: —

A weighed amount of leaf was ground thoroughly with a weighed amount of acid-washed sand and a certain volume of double distilled water, and the whole washed into the apparatus with sufficient double distilled water to bring the volume up to the standard volume used in the particular series in question. This, of course, gave to each flask a standard constant dilution value. To this mixture was then added a like volume of 1 per cent. solution of Merck's perhydrol, thus making the H_2O_2 concentration of the total mixture .5 per cent. The amount of oxygen liberated in ten minutes was arbitrarily taken as the measure of enzyme activity. Several different forms of apparatus were used, but for large amounts of leaf any ordinary water displacement method was found to be very satisfactory. (Care should be exercised where this mode of analysis is used, to take into account the absorption of oxygen by the water.) In making determinations where the amount of material was very small, the apparatus designed by Lohnis for use in milk examinations was found to be more convenient. Practically all determinations were made at temperatures ranging from 17° to 23° C. The action of the catalase is much accelerated by shaking, as pointed out by Loew, and each test was shaken under exactly similar conditions in all the determinations made. It was found necessary to use this method for the determination of the catalase activity, as any method involving titration, such as the permanganate method, was unsatisfactory, due to the reaction of certain constituents in the tissue with the reagents.

Table VI. shows the relative amounts of oxygen developed in normal tobacco leaves, and it is to be noted that the catalase of the dark green leaves was much more active than that of the light green leaves. This was found to hold true, to a certain extent, for light and dark green leaves even on the same plant. The basal leaves of older plants, which in some cases were almost mature, and of a lighter color than the middle and upper leaves, developed in every case relatively less oxygen. This was particularly true in the case of Havana tobacco. Broadleaf did not show such a wide divergence, but it should also be stated that in the Broadleaf plants employed in the determinations the basal leaves did not show any great color difference.

As will be noted, some of these experiments were made with plants grown under field conditions, but a greater number were made with plants grown in the greenhouse, under control conditions.

TABLE VI. — *Catalase Activity in Healthy Leaves.*

Weight leaf used=3 grams. Time of action=10 minutes. Temperature=17 to 23° C. Vol. of leaf+H_2O=100 c. c. Vol. 1 per cent. H_2O_2 added=100 c. c. g=greenhouse. f=field.]

Series.	Variety.	Amount of Oxygen developed (Cubic Centimeters).			Color of Leaf.	Number of Determinations.	Age of Plant.
		Maximum.	Minimum.	Average.			
A	Havana (g),	139.0	97.0	119.8	Dark,	40	Half grown.
B	Havana (g),	103.0	48.0	56.0	Light (basal),	26	Nearly mature.
C	Havana (g),	94.0	61.5	77.5	Light (whole plant light),	7	Half grown.
D	Broadleaf (g),	126.4	101.0	113.7	Dark,	3	Half grown.
E	Broadleaf (g),	154.0	119.5	126.3	Dark,	11	Nearly mature.
F	Broadleaf (g),	106.7	78.2	93.4	Light (basal leaves),	5	Nearly mature.
G	Havana (f),	176.0	115.5	124.8	Dark,	19	Half grown.
H	Havana (f),	147.6	93.1	100.2	Dark,	14	Nearly mature.
I	Havana (f),	121.0	72.9	91.0	Light,	6	Half grown.

These results show that the catalase activity varies somewhat even in healthy plants, dependent upon age and also, apparently, on the general condition of the plant. It shows clearly, also, that in plants of approximately the same age the catalase activity varies somewhat between plants with dark green leaves and those with light green leaves.

Even on the same plant this holds true, as can be seen from the results tabulated below.

TABLE VII. — *Catalase Activity of Light and Dark Leaves from Same Plant.*

[Plants nearly mature; procedure as in Table VI.]

PLANT No.	Number of Determinations.	Light Leaves, Cubic Centimeters of Oxygen developed (Average).	Dark Leaves, Cubic Centimeters of Oxygen developed (Average).
B_1,	4	51.8	119.8
X_{11},	3	62.0	125.5
104,	3	71.4	93.7
A_{17},	6	58.1	79.3

An examination and determination of the catalase activity in diseased leaves shows that the amount of oxygen developed is relatively much less than in the case of healthy leaves. In the table below are given some of the results obtained from diseased leaves. In these experiments the leaf tissue was used without reference to the light and dark areas of the individual leaf. It is significant that the activity is very much less than in healthy leaves. All the plants used in this series were badly diseased. It should be stated that in apparently mild cases of the disease the variation from the normal catalase content was not so great. The results shown here can hardly be compared with those given in Table VII., as the plants were not in some cases of the same age, nor were they grown at the same time.

TABLE VIII. — *Catalase Activity in Diseased Leaves.*

[Plants badly diseased; procedure as in Table VI.]

PLANT No.	Number of Determinations.	Cubic Centimeters of Oxygen developed (Average).
P_6,	8	47.2
R,	6	32.8
3_a,	9	54.5
A_x,	11	69.6
Total,	34	51.0

In the next table will be found a comparison of the results of catalase activity in healthy and diseased leaves from plants grown at the same time and under identical conditions. The plants were inoculated artificially in as uniform a manner as possible.

TABLE IX. — *Catalase Activity in Leaves of Healthy and Diseased Plants of Same Age.*

[Procedure as in Table VI.]

LEAVES.	Number of Determinations.	Cubic Centimeters of Oxygen developed (Average).
Diseased,	10	52.3
Healthy,	10	119.0

The values here obtained simply substantiate those given in preceding tables, but in addition allow of a direct comparison.

The leaf tissue was used in the preceding experiments without regard to the light and dark green patches on the individual leaf.

It was thought that an examination of the light and dark green areas of individual leaves of mosaicked plants might give a clue as to whether the activities of the catalase were inhibited in one or both of these areas in comparison with a leaf from a healthy plant of approximately the same age and color.

It was found that the catalase activity of the dark green areas approached that of the normal leaf of the same color, while the catalase activity of the light green areas was much below normal, even in the case of a light green normal leaf being used for comparison. The values obtained are given in Table X.

TABLE X. — *Catalase Activity in Diseased Leaves.*

[Comparison of light and dark green areas; procedure as in Table VI.]

SERIES.	Number of Determinations.	CUBIC CENTIMETERS OF OXYGEN DEVELOPED (AVERAGE).	
		Light.	Dark.
X,	4	42.1	73.6
0,	3	37.0	95.4
21,	8	54.3	103.0

Diastase. — It is a well-known fact that diastase is intimately connected with metabolism in the leaf in practically all chlorophyll-bearing plants, as well as in many of the fungi, and the relations of the activities

of diastase in the mosaic disease are of rather significant import, as can be easily shown. It was pointed out several years ago by Woods (*loc. cit.*) that the action of oxidizing enzymes when present in solutions containing diastase tended greatly, under ordinary conditions, to inhibit the activities of the diastase. Turning more particularly to the mosaic disease, he made the observation that in the cells of the light green areas, although they formed starch practically in a normal manner, so far as could be observed the starch was not translocated, and that in the morning there was practically as much starch present as at night, which is not the case in a normally functioning leaf. In this case it was found that practically all the starch disappeared in the night and was translocated.

Recently there has been more or less contention as to the exact method of action of diastase on starch, and within the last two or three years important investigations have resulted in the opinion, substantiated more or less in detail, that the diastase of the older writers is not one enzyme alone, but is made up of at least two components. The first of these breaks down the starches into, or as far as, the erythro-dextrine and achro-dextrine stage, the second component taking up the action from this point and completely hydrolizing the starch to the sugar compounds which are found to be present, as the next step in the process of metabolism.

It was in the light of these investigations that the writer took up the question of the diastase activity in the mosaic disease, and it was found to be less active in the leaves which showed severe symptoms of the disease than in those which showed only a slight trace. There was, however, apparently a greater or less breaking down of the starch in all the leaves examined, so far as could be determined by the colorimetric methods, which, although not altogether satisfactory, may be relied upon as much as any of the present-known methods of determination. At the morning examinations the starch did not in some cases take on the color of the normal starch in the healthy leaves, but was accompanied by a yellow brown to a reddish or violet coloration, dependent somewhat on the strength of the indicator used. The strength of the iodine solution used in this case was a fiftieth normal iodine-potassium iodide solution. This reaction would indicate that the starch to a certain extent had been acted upon at least partially by the diastatic enzymes, and would indicate also that it was possibly the first of the components above mentioned which was more active, and that the second was more or less inhibited in its action. In the normal leaf, of course, there was a certain amount of starch present indicated by the blue coloration of the granules. The amount was slight, however, compared to that in the diseased leaves, and in no case was there any of the brown or violet color, almost complete hydrolysis having apparently taken place very rapidly. This would indicate, as pointed out by Woods, that the oxidizing enzymes, of which we will make mention, and which are present in excessively large amounts in the diseased areas of the leaf, do play an important rôle in the controlling or inhibiting of the activities of the diastatic enzymes, but not on the

diastase in the old conception of the term. Rather it might be said the action is on the primary enzyme concerned in diastatic activity, if the newer concept of diastatic activity above advanced is true, as it would seem to be from the unpublished investigations of Roessler of the University of Prague, who was able to separate by salting out from a very carefully prepared solution of the ordinary diastase at least two components having the respective actions above mentioned. In no case, as indicated by the color reaction obtained, did we get a complete hydrolysis of a large amount of starch, the process only being carried on, apparently, as has been indicated, — as far as the erythro-dextrine and achro-dextrine stage. It was attempted in our experiments to isolate or rather separate out diastase in a more or less pure form from the leaves of healthy and diseased plants, and although certain results were obtained, it was rather a difficult matter, as in the writer's experience it has been found that diastase is one of the most difficult of the enzymes to purify to any extent. The protective colloids, etc., during the purification are separated away from the enzyme aggregate, and the purer ferment becomes less active. The reason for this cannot be very well explained at the present, but it is the experience of all investigators with diastase that this is a fact. However, results were obtained which seemed to indicate that the diseased leaves contain relatively less "diastase" than do the normal healthy leaves.

Chlorophyllase. — This enzyme has been found to be always present with chlorophyll in amounts directly proportional to the amount of chlorophyll present, and according to Willstätter and Stoll[1] does not bring about an hydrolysis but an "alcoholysis,"

$$RCOOC_{20}H_{39} - C_2H_5OH \;.\; RCOOC_2H_5 - C_{20}H_{39}OH$$

in the presence of ethyl alcohol. It forms the alcohol phytol, $C_{20}H_{39}OH$, from the radical in the presence of ethyl alcohol and not water only.

Very little is known about its action in the plant cell, and although the writer was able to demonstrate its presence in both healthy and diseased leaves, no quantitative data were secured as to its relative activity in healthy and diseased tissue. Until better methods are worked out for its purification and rapid determination it would be futile to hazard an opinion in regard to its specific action in the cells of healthy and diseased leaves.

Oxidases and Peroxidases. — Woods (*loc. cit.*) was one of the first to observe that in mosaic-diseased leaves the oxidase activity was greatly increased. Since then it has been found that in the curly dwarf disease of the potato and sugar beet the oxidase activity is greatly increased in the diseased leaves as compared with that of the normal. These two diseases have been for the most part regarded as physiological, and it is

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[1] Willstätter and Stoll: Unt. über Chlorophyll XI und XIII. Über Chlorophyllasa. Liebig's Ann. der Chemie., 378, 18 (1910); 380, 148 (1911).

a significant fact that this excessive activity of oxidizing enzymes has been more frequently noted in diseases of this character than in those which are caused by bacteria or fungi. The reaction of the host is apparently different in the latter case.

Bunzel[1] has noted that the oxidase activity varies with the age of the plant in the curly dwarf disease of potato, reaching its greatest activity when the plant growth ceases.

The writer has also found this to be true for tobacco to a certain extent, and always met with greater activities of the oxidases as the leaves were approaching maturity. This was marked in the case of normal plants, but not so much in the case of diseased leaves.

In the writer's examinations of healthy and diseased tissue not only qualitative colorimetric methods were employed, but also a simplified Bunzel's oxidase apparatus was made use of. This has been found to be the most satisfactory method for the quantitative estimation of oxidase activity.[2]

A few of the quantitative results obtained are given in Table XI.

TABLE XI. — *Oxidase Activity in Normal and Mosaic Sap.*

[Manometer readings in centimeters of mercury. Bunzel apparatus mod.]

Experiment.	Time in Minutes.	Normal.	Diseased.
A,	0	0	0
	30	−0.60	−0.80
	60	−1.09	−1.23
	75	−1.12	−1.29
	120	−1.22	−1.43
B,	0	0	0
	30	−0.32	−0.50
	60	−0.80	−0.70
	75	−1.02	−0.96
	120	−0.92	−1.21
C,	0	0	0
	30	−0.51	−0.46
	75	−0.63	−0.88
	100	−0.70	−0.91

It will be noticed that the mosaic sap is higher in total and average in every instance.

For the qualitative determinations the usual guaiac test was employed. The guaiac test for oxidases and peroxidases is too well known to require

[1] Bunzel, H. H.: Oxidases in Healthy and Curly Dwarf Potatoes. Jour. Agr. Research, Vol. II., 5, 373–404 (1914).

[2] Bunzel, H. H.: The Measurement of the Oxidase Content of Plant Juices. U. S. D. A., Bur. Plant Ind., Bul. No. 238 (1912).

an extended explanation. The results obtained by this method in every case showed the diseased leaves to contain much more oxidases than the healthy ones of the same age; this was also true for peroxidases, but here, of course, the reaction with guaiac was somewhat masked owing to the presence of the oxidases and their reaction.

In examinations of the roots of healthy and diseased plants the same condition was observable; there was always an excessive activity of the oxidizing enzyme to be noted.

In going over the results of the experiments with the enzymes in question, the main point brought to the attention is that there is in all diseased plants an excessive activity of the oxidizing enzymes, and a corresponding decrease in the activity of the diastatic enzymes and catalase. This at least indicates a very much disturbed equilibrium and a consequent derangement of normal function on the part of the cells. Naturally the ones most affected by this disturbance are the dividing or meristematic cells, as these are the cells upon which the plant is dependent for its subsequent growth, and any deviation from the normal is more likely to be indicated in the development of these cells than in those of the other parts of the plant. Any change in function induced here will leave its imprint to a greater or less extent on the cell during its subsequent existence, hence the peculiar manifestations of the disease in the leaves.

It is true that plants attacked by parasites sometimes show an excessive activity on the part of certain enzymes, but, as a rule, the disturbance is more local in its nature. It is also a fact that malnutrition, such as partial starvation, drought, etc., will bring about an excessive production or activity of the oxidizing enzymes in particular, as has been pointed out by Bunzel, of general distribution throughout the plant; but this, except in cases of maturing plants, changes upon restoration of normal conditions, and tends to become normal.

Reaction of Mosaic Sap with Various Substances.

We have seen that the enzymatic activities of the plant are very much disturbed in disease; also that it has been impossible to demonstrate the presence of any forms of bacteria or fungi either in the tissues themselves or in the expressed juice.

It is a fact, as shown by practically all investigations, that the disease is very infectious. This fact alone in the minds of many is sufficient to place the causative agent among the parasitic organisms. The field, however, is limited to that class of organisms designated as "ultramicroscopic" organisms, about which very little is known, and in the case of plant diseases not even a semblance of the demonstration of the activities of such organisms has been made.

Owing to the fact that the enzyme activities are much changed, as has been demonstrated in the preceding pages, and also to the fact that not only the activities of the oxidizing enzymes are changed, but also the

activities of others; it was believed by the writer, with Woods and others, that the disease might be physiological in nature, particularly in so far as the causal agent, not being a living organism in the ordinary conception of the word, was concerned.

So little is known about the action of the so-called ultramicroscopic organisms that it is an open question in the writer's mind whether this division should be the dumping ground for all infectious diseases about the etiology of which little or nothing is known.

It is conceivable that other causes, not organic in nature, may be able to produce the manifestations of parasitism. Under this type of infection would be included infectious diseases caused by enzymes or the resultant product of the activities of a group of enzymes.

Certain reactions of the juice from diseased plants tend to confirm this view, and in the following pages are given the results obtained by the writer and other investigators relating to the reactions of these juices with various reagents.

Drying. — It has been shown by various investigators that the dried leaves of the mosaic-diseased plants retain their infectious qualities for a long time. Beijerinck and Allard found that diseased leaves were capable of causing infection after being dried for periods of two years and eighteen months, respectively. The writer has used material three years old, and obtained infection in a great majority of cases. The results obtained are given below.

TABLE XII. — *Air-dried Mosaic Leaves, finely ground and macerated with Cold, Distilled Water.*

[Leaves (herbarium specimens) three years old.]

Number of Plants inoculated.	POINT OF INOCULATION.	Number of Plants infected.	Per Cent. Infection.
10	Below terminal leaflets, ...	10	100
12	Main stem near base, ...	11	91
7	Midribs of a basal leaf, ...	6	86
13	Midribs of a basal leaf, ...	12	90

Filtration. — The use of various filters such as the Chamberland, Berkefeld and Kitasato types, as a means for the separation of bacteria and other organisms in a fluid, has been widely adopted in recent years, and more recently filters possessing different sized pores have been used for differential diagnostic purposes in work on the so-called "ultramicroscopic" organisms, enzymes and toxins. While these methods are without doubt of importance, it should always be borne in mind that to obtain true filtration effects comparatively large volumes of the fluid should

be used, otherwise there is a strong possibility, particularly in the case of enzymes, that instead of a filtration occurring at once, a large amount of certain constituents may be adsorbed (dependent on the nature of the filter), and that true filtration may not take place until comparatively large amounts have been drawn through the filter. The writer has noted this particularly in work with enzymes, many of which are strongly adsorbed by various substances. Aside from the "ultramicroscopic" organisms, however, the bacteria cannot pass through many of these filters.

With reference to the causal agent in mosaic sap it has been found that it passes through both the Chamberland and Berkefeld filters, and even the finer grade of Berkefeld filter allows the passage of the causal agent. Beijerinck (*loc. cit.*) showed that the juice was still infectious after being passed through the Chamberland filter, and Allard (*loc. cit.*) and Clinton (*loc. cit.*) have both shown that the juice was infectious after passage through the Berkefeld (normal) filter. The results obtained by the writer agree with these observations, and also the juice was found to be infectious after passing it through the fine Berkefeld candle. The Kitasato filter was also used, and here positive infection was also obtained, although the percentage was small. The writer attempted to repeat these experiments with the Kitasato filter during the past year, but was unable to obtain the filter. In all cases relatively large amounts of the sap were used after filtration through paper.

The average percentage of infection obtained with each filter in the writer's experiments was as follows: —

	Per Cent.
Chamberland (average of 3 examinations, 1911),	91.0
Berkefeld (normal; average of 5 examinations, 1911),	63.0
Berkefeld (fine; one test only, 1914),	47.0
Kitasato (average of 2 examinations, not dated),	40.5

The work with the fine grade of Berkefeld and Kitasato filters should be repeated, but there are sufficient indications to warrant the insertion of these results at this time.

Resistance to Antiseptics. — The writer has at various times treated filtered and unfiltered juice with many of the antiseptics such as are commonly used to prevent bacterial action.

The following table contains the data and results obtained in one typical series of experiments of this character: —

TABLE XIII.

Antiseptic.	Amount of Sap used (Cubic Centimeters).	Period of Treatment.	Infection.
Toluol (2 c. c.),	10	2 months.	++
Toluol (2 c. c.),	10	4 months.	++
Chloroform (saturated at beginning),	10	2 months.	++
Chloroform (in excess),	10	2 months.	—
Chloroform (saturated at beginning),	10	4 months.	+
Chloroform (in excess),	10	3 days.	—+
Thymol (2 per cent.),	10	2 months.	+
Thymol (2 per cent.),	10	4 months.	+
Ether (saturated),	10	2 months.	+
Ether (saturated),	10	4 months.	+
Formaldehyde (1–4 H_2O, 1 c. c. added),	10	2 months.	—
Formaldehyde (1–4 H_2O, 1 c. c. added),	10	10 days.	—
Carbolic acid (5 per cent., 10 c. c. added),	10	2 days.	—
Chloralhydrate (½ mol.),	10	2 days.	—
Chloralhydrate (½ mol.),	10	20 hours.	—

++ = very infectious. —+ = one or two cases of infection, possibly accidental.
+ = infectious (over 40 per cent.). — = no infection.

From the preceding table it may be seen that the sap containing the causal agent of the disease varies greatly in its reaction to so-called antiseptics and other compounds. The writer[1] has already pointed out in a previous publication that the influence of certain capillary active substances on enzymes is very variable, aside from the specific toxic qualities of certain of these substances. In comparing the reaction of the sap containing the causal agent to certain of these compounds we find that there is a similarity of reaction to that shown by the enzymes. In the paper above cited it was shown that those compounds which changed the surface tension had, as a rule, dependent on their physical properties (hydrocolloidal or lipocolloidal), a certain definite effect on enzyme activities.

Taking up the discussion of the results in detail we find in toluol a compound which is not soluble in water to any great extent, and hence, behaving like a lipocolloid, having no effect on the action of the causal agent contained in the sap. Toluol, as a rule, has a more or less definite inhibitory action on living organisms.

Chloroform, when present in the sap not to exceed saturation, behaves also like a lipocolloid, as it is only very slightly soluble in the water, and

[1] Chapman, George H.: The influence of Certain Capillary-Active Substances on Enzyme Activity. Internat. Zeitschrift für Physik.-chem. Biologie., I Band, 5 u. 6 Heft (1914).

we find in this case that the activity of the agent is not destroyed. Chloroform in excess, however, does destroy apparently the causal agent of the disease. It is noteworthy that this action of chloroform exactly parallels that found to be the case with enzymes.

Thymol, when used in 2 per cent. concentration is very often used as a preventive to bacterial action, and also prevents the growth of fungi. We find, however, that when it is present in concentration not exceeding 2 per cent. in the sap the causal agent still possesses its infectious qualities for some time.

Ether is a substance which, like chloroform, has lipoid-like properties, but which has a definite action on the surface tension, lowering it considerably. Sap containing ether to the saturation point, which lowers the surface tension from 1 to about .619, was still infectious four months after treatment, although the percentage of infection was much decreased.

A solution of the sap containing approximately .8 per cent. of actual formaldehyde was very injurious, and at the end of two months no infection was obtained. At the end of ten days in one experiment, however, plants were inoculated and two cases of mosaic disease developed from a series of eight plants, but it is believed that this may possibly have been an accidental infection, as in no other instance was infection obtained. In formaldehyde, however, we have a compound which has a specific narcotic action on certain enzymes aside from its surface activities.

Where carbolic acid was added to a solution of the sap the active principle was apparently destroyed.

In chloralhydrate we have a substance very soluble in water, but not possessing any relatively great surface activity. It has, however, a specific toxic action on the causal agent of the disease, and even after twenty hours no infection was obtained. These results with chloralhydrate are in complete accord with those obtained in the enzyme work previously mentioned.

Most of the substances used in the above experiments possess a very definite toxic action to all organisms, particularly bacteria and fungi. As to their effect on the so-called ultramicroscopic organisms the writer is unable to state, not having had the opportunity of working with so-called cultures of these organisms. The parallelisms between the surface-tension effects of these substances on enzymes and on the sap containing the active principle of the mosaic disease are very striking.

Having shown that the causal agent is not bacterial or fungous in character, we must eliminate for the present the supposition of the presence of a toxin or virus in the pathologist's conception of these terms, as it is usual to conceive of these substances as being either the product of an organism or the activity manifested by the organism itself. As to the production of toxins and viruses by the so-called ultramicroscopic organisms little is known. Noguchi was the first to apparently demonstrate that such organisms do exist, and was able to cultivate an organism obtained from the brain of patients suffering from infantile paralysis.

However, these organisms were always mixed with certain bodies probably of a protein nature, and Noguchi, himself, so far has been unable to state absolutely which may be the active agent, although he naturally infers from his inoculation experiments that the organisms found must be the causative agent owing to the extreme infectious character of the disease. He, however, states that it is not absolutely clear to him whether the organism alone or a combination of this organism with the bodies found in culture associated with it are capable of producing infection. He does state, however, that in the case of *animal* pathology no such symbiotic relationship has so far been observed. From the character of his statement, however, it is clearly indicated that he does not preclude the possibility of such a condition arising.

Probable Character of the Causal Agent.

The question as to the exact character of the causal agent of mosaic disease has been an extremely interesting one to investigators, and studies on this phase of the problem have narrowed the field by the elimination from consideration of fungi and bacteria, as has previously been shown not only in this work, but also by many other investigators. This also precludes the presence of a virus or a toxin resultant from the activities of such organisms.

This leaves, then, for consideration as the causal agent an "ultramicroscopic" or "invisible" organism and the enzymic activities in their fullest conception. The reactions of the so-called "ultramicroscopic" organisms are little known at present, and about the only grounds for admitting of such a class of organisms is the *infection* factor, and possibly *reproduction* to a certain extent. We do know, however, many reactions of the class of substances called enzymes and toxins, but fundamentally the differentiation of the three above mentioned is difficult, and is perhaps in many cases impossible. Working with filtered sap from mosaic-diseased plants, we get the following results in comparison with reactions of some of the so-called "ultramicroscopic" organisms and toxins.

Temperature. — The sap containing the causal agent of mosaic disease becomes non-infectious; in other words, becomes inactive when heated to about 80° C. for a short time. It is reported that ultramicroscopic organisms and toxins are killed or rendered inactive, respectively, by exposure to heat for any length of time at temperatures somewhat below 100° C. Enzymes are also rendered inactive at temperatures somewhat below 100° C. All three react practically alike as regards temperature. The causal agent in mosaic sap, as may be seen, is also rendered inactive at temperatures below 100° C.

Size. — As to size, nothing can be definitely stated, but it is a fact that the ultramicroscopic organisms, enzymes and toxins must have a diameter of less than .1 μ, otherwise they would become visible under the higher powers of the microscope. In no case has it been possible to demonstrate the presence of organisms under even the highest powers available.

Reaction to Antiseptics. — It is stated that the ultramicroscopic organisms are not, to any extent, affected by the ordinary antiseptics, and the same is true for toxins in general. On the other hand, enzymes and their activities are very strongly affected by the substances usually made use of as antiseptics, and this is found to be true, with one or two possible exceptions, in the case of mosaic sap. It has been shown that formalin, carbolic acid, chloralhydrate, and even chloroform in excess, will inhibit the activities of the causal agent in mosaic sap, while, on the other hand, such substances as ether, toluol, thymol and chloroform in dilution have littlé or no effect. While all three classes are to a certain extent affected by antiseptics in general, the enzyme group is most strongly affected, and in the case of the mosaic we find this reaction; also, as has been pointed out, the effect of substances possessing marked surface-active properties is, in the case of mosaic sap, quite analogous to that of these substances on enzymes. It had been hoped to carry on more detailed work on this point, but as yet no opportunity has offered to take up this phase of the matter. Allard[1] has studied the effects of alcohol, ether and other substances on mosaic sap, and an interpretation of his results, with particular reference to the surface-active properties of the substances under consideration by him, parallel the author's findings in the case of enzymes to a marked degree. It is believed that more work of this character might throw considerable light on this matter.

Activity. — So far as can be judged from laboratory results the activity of the causal agent in mosaic sap is continuous, and as this holds true not only for organisms but, within limits, for enzymes and toxins as well this property cannot be made use of for differential purposes.

"Koch's Laws" or "Postulates," so called, are followed by all three of the classes under consideration, and the same is true in the case of mosaic disease; the causal agent obeys these laws, and might well be placed in any one of the classes so far as this property is concerned.

The Kitasato filter has been used by some as a means of separation of "ultramicroscopic" organisms from enzymes and toxins, and although the arbitrary use of any one filter as a standard, unless the size of pores, adsorption properties, thickness of walls, etc., are carefully taken into consideration, may be open to question, this procedure has been followed in some instances in animal pathology, and it has been found that the Kitasato filter held back the organisms and that no infection could be obtained from the filtrate. In the case of the mosaic disease, however, we find that apparently, as has been previously indicated in this paper, where large volumes are used, the causal agent passes through the Kitasato filter, and we do get infection from the filtrate.

The disease is infectious, but whether the infection may be indefinitely transferred through several plants with undiminished virulence is open to question. On some varieties of tobacco this does not apparently take

[1] Allard, H. A.: Some properties of the virus of the mosaic disease of tobacco. Journal Agr. Research, Vol. VI., No. 17 (July, 1916).

place, but so far as the writer's observations go the virulence of the causal agent is not lessened appreciably. This property is one of the strongest points advanced by those favoring the theory of the presence of a definitely organized parasite as the causal agent of the disease. It is, however, entirely possible that enzymes or similar substances introduced into a plant even in extremely small quantities, are capable of regeneration of a certain kind, and indeed it is held by some that enzymes do *grow* and even reproduce themselves under certain conditions. The difficulties encountered in the study of this phase of enzyme work are very great, however, and it is questionable if such statements can be as yet definitely accepted.

Organisms, even of the ultramicroscopic class, in their reactions would not follow the law of proportionality, but in the case of mosaic sap and its reactions we find, by measuring the relative activities and reactions of the enzymes present that apparently a proportionality of reaction for any one lot of sap does hold. The writer has very often found in the measurement of the activities of the catalase and oxidase particularly that not only a fairly definite relation exists between the various enzymes, but that reaction of any one is dependent on the amount of sap used. Of course, here we are dealing with a mixture, and it may be open to question if the measurement of the enzyme activities is a true measure of the activities of the causal agent.

The whole subject of the differential diagnosis of enzymes, toxins and ultramicroscopic organisms is an extremely difficult one, and no sharply dividing lines can properly be drawn between them. It would appear to the writer that in some cases, at least, it is entirely dependent on the viewpoint and interpretation of the investigator as to the class to which certain diseases should properly be ascribed.

The factors of reproduction and infection, as ordinarily understood, have proved a stumbling block to the acceptance of the idea that there may be other forms of matter aside from organisms capable of reproducing a disease, but there is in reality very little real ground for taking this attitude. In the case of the mosaic disease there are certainly many reactions which will not allow of placing the causal agent in the class of ultramicroscopic organisms. The general distribution of the causal agent in a diseased plant, its exceedingly localized action on the meristematic tissues, this action being apparently confined to the nascent chlorophyll, the non-uniformity of response to apparently favorable conditions during any one season even on one field, and also its individualism as shown by plants growing together (one often diseased and the other not) are to the writer indicative of something of a different character.

It is also possible that in the search after the infinitesimal the fact that a highly organized plant as a whole may react in the same manner as some of the simpler organisms has been overlooked. It is as a rule not the presence of an organism alone which is responsible for the manifestations of disease, but the products of the metabolism of the organism.

If the metabolic processes are changed ever so slightly, due to any stimulus, far-reaching effects may be induced throughout the organism, and this we find to be the case in the mosaic disease, and the writer believes that it is justifiable to look upon the matter in this light, as it is no more hypothetical than the concept of an "ultramicroscopic" parasite, which, if demonstrated (and no amount of concentration or methods of culture have indicated in any way the presence of aggregates or colonies), certainly would become visible if multiplication occurred.

Theoretically is it possible to conceive of an organism, functioning as such, to be made up of so few molecules of protein, fat and carbohydrate that it would be impossible to demonstrate its presence? If so, our ideas of relative size of molecules of protein, etc., must be changed.

Prevention and Control.

The question of the prevention and control of mosaic disease is of prime importance to the grower, entirely aside from more technical considerations as to the exact cause or causes of the disease, and it is believed that with reasonable care it is possible for the grower to lessen materially the amount of mosaic in the field.

Many recommendations have been made regarding treatment of diseased plants after they have once contracted the disease, but so far the writer has never observed a plant which, once attacked by the disease, recovered at any subsequent period of its growth. On the other hand, it has never been observed that the disease killed a plant, at least in this region.

It is doubtful, owing to the character of the disease, if it can ever be entirely eliminated on some soils and under certain unfavorable conditions occurring during some seasons. As has been indicated previously there is apparently little or no relation to be found between excess or lack of food materials and the prevalence of the mosaic. It has been in some instances stated that favorable results have been obtained from the use of lime in different forms, but this treatment cannot be recommended for various reasons. Experimentally it has been shown that heavy liming has little or no effect on the disease once a plant has contracted the disease, and even when applied to soils from old beds no consistently favorable results have been obtained (see page 91). Used in the larger quantities it might be inferred from the results that the lime apparently did exert a beneficial action, but to apply lime generally in such amounts would be folly, as it would in many cases bring the soil to a comparatively neutral or alkaline condition, which reaction would favor the development of root rot, caused by the fungus, *Thielavia*, and this, once thoroughly established, in a field or seed bed, is much more injurious to tobacco than is the mosaic disease.

As has been pointed out, the writer, from his observations, is strongly of the opinion that much of the field infection may be traced to the seed

bed, and as a rule those beds which have long been used or carelessly handled are found to be producers of mosaicked seedlings in far larger numbers than are found on new beds or on beds which have been carefully sterilized either by steam or formalin.

It has been found that the soils of old beds do tend to produce more mosaicked plants than do those of new beds, although it may be possible that under field conditions the differences in amount during different seasons may vary. Soils brought into the greenhouse gave the following results: —

TABLE XIV. — *Experiments with Soils from Old and New Beds.*

[Seedlings transplanted in sterilized soil.]

Soil.	Number of Seedlings transplanted.	Number Diseased Four Weeks after Transplanting.	Diseased (Per Cent.).
Soil A (old bed),	60	45	75.0
Soil 21 (old bed),	43	17	40.0
Soil I*a*,	50	21	40.0
Soil B (new bed),	30	3	10.0
Soil C (new bed),	49	2	4.0

The soil from the old beds was in very bad condition and had been very carelessly handled, apparently.

A count of mosaicked seedlings left in these old-bed soils six weeks after the transplants was taken, showing, respectively, an infection of A, 43 per cent.; 21, 32 per cent.; I*a*, 17 per cent.; B, 6 per cent.; and C, 7+ per cent.

It is evident that some of the seedlings were infected during transplanting, probably by handling diseased seedlings and then healthy ones, thus transmitting the disease. This method of transmission at the time of transplanting is very common, as has been pointed out repeatedly.

It has been shown that much of our infection may originally come from the seed bed as a result of the soil becoming infected for any reason. The use of tobacco stems and tobacco water has also been found by many investigators to cause infection. The amount of infection resulting from watering beds with water extract of diseased stems is, however, problematical, and it is not believed by the writer that this is an important factor in mosaic transmission, especially if the stems are steeped in hot water. The broken, decaying roots of diseased plants left in the beds also carry the causal agent of the disease as do the stems of diseased plants, and freezing has apparently little or no effect on it, so the use of stems on the seed bed should be carefully attended to in order not to apply any from diseased plants. Where stems and tobacco water are applied year

after year without attention to this point the bed usually becomes more seriously infected.

One of the cheapest methods for the control of this disease in the seed bed, where it can be advantageously carried out, is to change the location of the beds to soil on which no tobacco has been grown, and to avoid the use of stems and tobacco water. Occasionally, however, some slight infection will occur even here, but as a rule not to any great extent. If proper attention is paid to watering, ventilation, etc., little trouble of this character is to be expected in new seed beds.

It has been shown in Connecticut and elsewhere that a thorough sterilization of the seed bed by steam at a boiler pressure of from 70 to 90 pounds is also a satisfactory method for the control not only of fungous diseases but weeds also, and the same holds true for the mosaic disease. The writer has seen this tried a number of times with excellent results where the above-mentioned pressures have been used. Some growers, however, seem to be of the opinion that the prime value of steaming is to kill weed seeds, and so use low pressures. While low pressures will kill weed seeds, it is questionable if they will sterilize the soil sufficiently to kill the spores of fungi or render inactive the causal agent of the mosaic, although under laboratory conditions it is rendered inactive at temperatures of about 80°C, equivalent to 176°F. In some of our experiments conducted some years ago it was strongly indicated that improper partial sterilization would not entirely rid the soil of the causal agent of mosaic.

It might be stated here that, in many cases where the growers have reported failure in the control of diseases after steam sterilization, inquiry has usually brought out the fact that too low pressure was used, and as a result thorough sterilization was not obtained. Another source of failure of beds after sterilization with steam, under high pressure, has been that the grower has not paid sufficient attention to watering. This matter should be closely attended to, as a sterilized bed, particularly on light soils, dries out very quickly, and needs much more attention than is usually given a bed under ordinary conditions. If the watering is neglected there is very often a severe checking of the germination of the seed, and in some cases a partial loss of the bed.

Formalin sterilization may also be used, and is quite as satisfactory, especially when used on light soils. On heavy soils it is not quite so convenient to apply, however. Where formalin is used the beds cannot be sown until all the formalin is out of the soil, which usually takes from ten days to two weeks. This very often is too long a delay, particularly where spring sterilization is practiced.

It has been pointed out that the workmen may be a rather important factor in transmitting the disease (page 88), and in cases where at transplanting time diseased seedlings are handled it has been recommended by Clinton[1] that the hands be thoroughly washed in soap and water

[1] G. P. Clinton: Chlorosis of Plants with special reference to Calico of Tobacco. Conn. Agr. Exp. Sta. Rept., 1914, p. 417.

before again handling healthy seedlings. If these precautions are taken, according to Clinton, a considerable amount of mosaic infection will be avoided at the time of planting.

It has been repeatedly shown that care should be exercised during early cultivation not to cut the roots or touch broken or abraded leaves of plants and then subsequently touch other plants, for the disease is very easily transmitted in this way, as the fine hairs or epidermis may be broken and infection occur. The amount of infection due to cultivation is, however, in the writer's opinion, slight, but as much care as is commensurate with efficiency should be exercised by the workmen during cultivation.

The advisability of the removal of diseased plants is open to question, and on the whole it cannot be economically recommended unless the plants can be replaced early in the season. As has been previously pointed out, the disease may be carried from plant to plant when topping, etc., and the subsequent sucker growth will become mosaic. At this time, however, the commercial leaves are of such size that their value will not be materially impaired, but if possible, to prevent a certain amount of infection, only healthy or diseased plants should be topped at any one time. Of course, all suckers developing later, diseased or otherwise, should subsequently be removed from all plants, not only for the sake of the commercial leaves, but to prevent a ragged looking field, giving the appearance of a large amount of mosaic.

It has been very difficult to associate any particular type of soil with general occurrence of mosaic disease, but on the whole, from data gathered at different times, the heavier types of soil in the valley appear to be more generally favorable for the production of mosaic-diseased plants. This cannot be definitely stated, however, as the data are complicated by the fact that in some cases, on both heavy and light soils, the condition of the soil as regards organic matter present enters into the question. The writer has observed that on many heavy soils where comparatively large amounts of organic matter are present during certain seasons, in comparison with similar soils deficient in organic matter, the mosaic is much less. To a certain extent this holds true also for the lighter soils. The exact relation existing between the mosaic disease and these factors is at present not enough studied to warrant definite conclusions, but Sturgis (*loc. cit.*) was of the opinion that clayey soils were favorable to its production. It is a significant fact that many of our tobacco soils are somewhat deficient in organic matter, however. Well-cultivated and consequently well-aerated soils do not apparently produce as many mosaicked plants as those which are not well cultivated.

Another factor which should be carefully attended to is that of the moisture conditions in the bed at the time the plants are pulled. It should not be too moist nor too dry, as in either case the roots are apt to be broken and infection from handling result more certainly than when the plants are removed with a minimum of root injury.

SUMMARY.

1. The mosaic disease is not caused by fungi or bacteria. It has never been possible to demonstrate the presence of these organisms in the tissue of any part of the plant.

2. The disease is highly infectious, particularly when inoculated into young plants, all subsequent growth exhibiting marked symptoms.

3. The disease is not contagious.

4. Until more is known about the action of the so-called "ultramicroscopic" organisms, the disease cannot be ascribed to an organism of that class, as the character and reactions of the causal agent do not in many respects coincide with reactions of that class of organisms.

5. Many of the reactions of the causal agent are of such a nature as to indicate that it is either an enzyme, an aggregate of enzymes, or the product of enzyme activities.

6. The enzyme activities of diseased plants are greatly altered, far more than is usually the case in plants which are attacked by pathogenic fungi or bacteria.

7. As a result of the writer's experiments, it is believed that the disease is primarily induced by a disturbance in the enzyme activities and their relation to each other, due to abnormal metabolism, and not by any parasite.

8. The pathogenicity of a disease is not necessarily a proof that it is of parasitic origin, as it is conceivable that similar conditions may exist relative to enzyme activities, although the extent of such action is not known at present.

9. On fields where the mosaic disease is prevalent, the primary infection can usually be traced to the seed bed, and many healthy seedlings are infected by the workmen when setting the plants. It is estimated that about 80 per cent. of the infection occurs in this manner.

10. Owing to the nature of the disease the matter of absolute prevention and control is difficult, but with careful attention to details of sterilization of the seed bed, and handling of the plants at time of transplanting, a large percentage of infection may be avoided.